ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL CANCER INSTITUTE

FISCAL YEAR 1972
PART II

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT

of

PROGRAM ACTIVITIES

U.S. NATIONAL CANCER INSTITUTE

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Fiscal Year 1972

Part II

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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

July 1, 1971 through June 30, 1972

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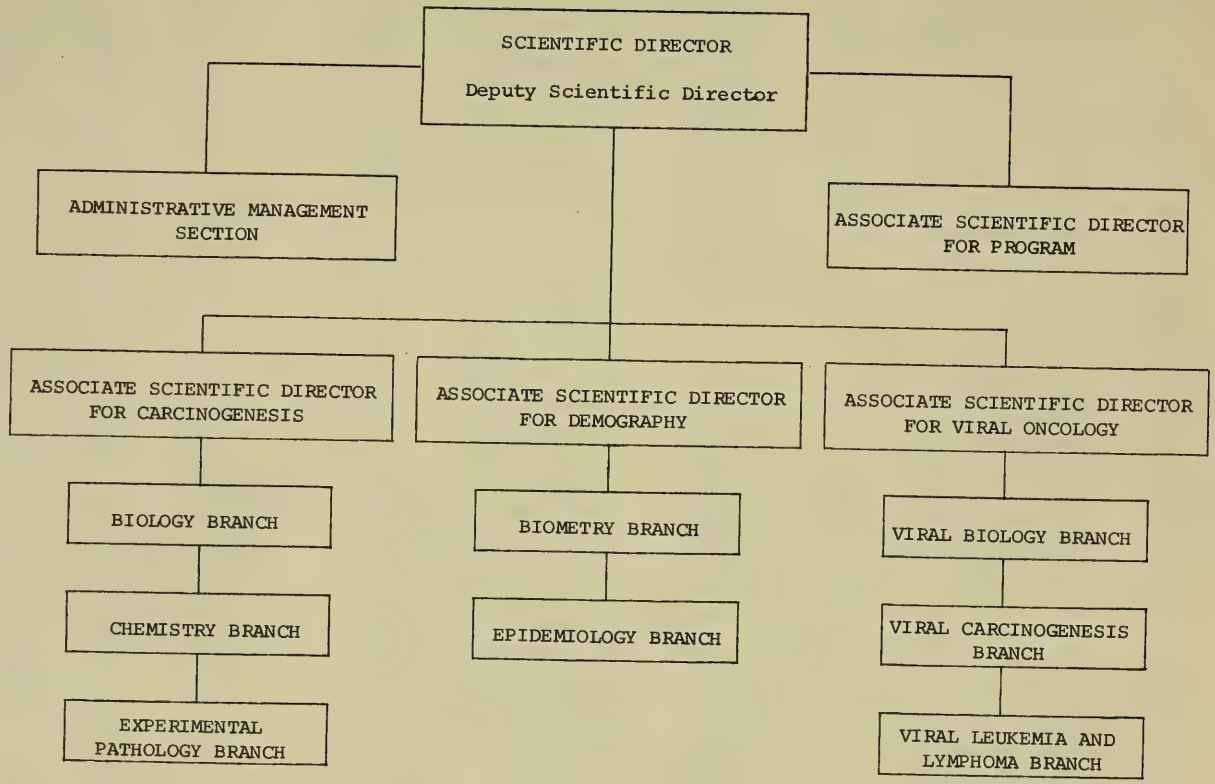
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SUMMARY REPORT
ETIOLOGY AREA - NATIONAL CANCER INSTITUTE
July 1, 1971 - June 30, 1972

For the last three years the summary report of the Scientific Director for Etiology dealt with general aspects of the total Etiology program. The report set forth goals and objectives of the Area, a conceptual base upon which a broad attack on cancer etiology and prevention could be developed, program approaches to be used for attaining the objectives, and indicated the magnitude of the task and the investments required. These features were set forth in broad perspective and a fairly long time frame: the material contained therein is still germane, and will not be repeated here.

A simplified synopsis of major goals, and of pathways to them, is given in Figure 1. More detailed program logic networks for Demography, Carcinogenesis and Viral Oncology will be found under specific area presentations.

All activities in Etiology are directed towards cancer prevention. Research output is continually utilized in resetting more advanced priorities and in formulating those activities that offer the highest promise of success. The major structures of this scientific management process are indicated in Figure 2.

Newly enacted legislation and funding for a National Cancer Program pose novel challenges and opportunities in research and for vigorous cancer control activities in man. This legislation and program recognize the promising advances of cancer research which now make it possible to formulate direct intervention in man's behalf: the definition and engineering of a safer environment, and the identification of those individuals who need increased attention because of their increased risk to develop cancer.

From now on, the Etiology area will be increasingly committed to cancer control activities. Research will be equally emphasized, as the continuing success of control measures in man will depend almost exclusively on the successful research of the past and future.

IMPACT OF CANCER

Cancer occurs in all parts of the world, but variation in the incidence of specific forms of cancer is the rule rather than the exception. A range of ten or twenty-fold is common and for some types of cancer it is far wider.

In general, the rate of cancer mortality in the United States occupies an intermediate rank in the worldwide range, but the rank of mortality from specific types of cancer varies markedly. The United States white population has the lowest mortality from cancer of the stomach and close to the highest from cancers of the colon and female breast. As shown in Table 1, the incidence rates of various cancers in the U.S. are expected to increase from a low of 25% each for leukemias and breast cancer to a possible high of 180% for cancer of the pancreas. This table also presents leads towards identification of etiologic factors and towards prevention.

Available evidence suggests that environmental agents and social practices, rather than conventional genetic factors, (except -- see discussion "Huebner-Todaro" oncogene, below), are largely responsible for variation in the occurrence of cancer in different populations. *It should therefore be possible to reduce the occurrence of the major types of cancer in the United States to the level of the lowest ranking country for that type.* Such a reduction would cut mortality from cancer by one-third. This is the minimum goal we should aim for.

More than 52 million Americans now living will eventually have cancer (one in four persons) according to present rates. Cancer will strike over the years in approximately two of three families. In the 1970's in the United States alone, there will be an estimated 3.5 million cancer deaths, 6.5 million new cancer cases, and 10.0 million under medical care for cancer unless the present trends are moderated. Such trends mean that in 1976 there will be 346,000 cancer deaths and 747,000 new cases and in 2000, 471,000 cancer deaths and 1,085,000 new cases.

In the current year there will be 335,000 cancer deaths in the U.S. or one cancer death nearly every one and one-half minutes. There will be 635,000 new cancer cases and 975,000 Americans will be under care for cancer. The last full study on economic cost of cancers in the United States in 1962 estimated an annual cost at that time of $11 billion. About one-half the cancer cases are below age 65; life expectancy and economic earning power are reduced over a considerable portion of the economically productive ages.

Although cure rates for cancer patients have steadily improved over the past several years, the mortality (both in actual numbers and in rates corrected for population growth and other changes) continues to rise. This is because new cancer cases are appearing at an even greater rate than the improved rate of cures. Some kinds of cancer are increasing faster than others; by the year 2000, if present trends continue, cancer of the lung will have increased 52 percent, large bowel 40 percent, leukemias 25 percent and pancreas 180 percent to an overall total of 1.2 million new cases per year.

PROGRESS, PROBLEMS AND OPPORTUNITIES

A. DEMOGRAPHY. Cancer can be prevented by identifying its causes and removing them, and/or by increasing the body's defenses against them. Thus, it is important to know not only what causes cancer, but also who is unduly susceptible or resistant. Susceptibility may be immense. For example, if an identical twin develops leukemia during the first year of life, his co-twin is virtually certain to develop leukemia within weeks or months. This increased risk fades quickly with age, and entirely disappears by 6 years of age.

Recently there has been a substantial increase in our list of characteristics of persons who are unusually susceptible to leukemia, lymphoma and other cancers. Once such a list has been created, it can be examined for a common denominator. Many items can be explained by a known or possible genetic defect, sometimes extensive enough to cause various abnormalities in the appearance of chromosomes.

It should be noted that the only environmental agent known to be leukemogenic in man, ionizing radiation, and the strongest suspect among the chemicals, benzene, produce long-lasting chromosomal abnormalities in somatic cells.

By contrast, persons at high risk of lymphoma (solid tumors of lymph tissue) have inborn immunological deficiencies (ataxia-telangiectasia, Wiskott-Aldrich syndrome, or congenital x-linked agammaglobulinemia). Consistent with these observations is the increase reported in the occurrence of lymphomas among persons given immuno-suppressive drugs following renal transplantation. A sudden upturn in one form of lymphoma, Hodgkin's disease, at about 8 years of age, also suggests a relationship to immunological development. Normally, at this age, there is a marked spontaneous involution of lymphoid tissues throughout the child's body. Lymphoid tissues are centers of immunologic activity. It is tempting to think that some failure in this normal regression accounts for the upturn in the occurrence of the neoplasm.

There are many other inborn defects that carry high risk of cancer of various types. In the aggregate, they do not account for a large proportion of cancer but they probably do constitute a large percentage of cancers among young people. In this group, prevention or early detection and treatment can provide decades of healthy life. Peaks soon after birth in the frequency of diagnosis of leukemia, neuroblastoma, Wilms' tumor, and certain forms of brain cancer indicate that many of these neoplasms are likely to have been initiated during intra-uterine life.

Knowledge of these relationships permits physicians to examine children with certain defects more closely than usual, since early detection and prompt removal of these tumors can be life-saving. Scientists, on the other hand, can explore the origins of the tumors in the light of what is known of the associated birth defects. Thus, instead of studying a tumor in terms only of itself, new avenues of approach have been opened. What is learned through these rare mistakes of nature may well apply to prevention of large categories of cancer. In particular, the epidemiologic identification of individuals and populations at increased risk of different cancer patterns should aid the investigator in virology and chemical carcinogenesis in searching for specific etiologies or combinations thereof.

During the past year the following activities were begun or increased in emphasis:

(1) Migrant Studies - Theories of Cancer Etiology. The migrant studies have triggered extensive examination of some of the factors that may lead to colon cancer, involving laboratory studies and the examination of populations in different parts of the world. The epidemiologic evidence in colon cancer has led to an initiator-promoter model which is capable of being tested by collection of additional observations in man. A similar consideration of stomach cancer would imply that some very early events (before the age of 15) operate as initiators. For colon cancer the initiator would come later in life. The notion of early events having important effects much later in life is being studied with respect to breast cancer where there is now some evidence

that hormonal status within the first decade after puberty may be of considerable importance in the later development of breast cancer.

(2) Data Analysis - Death Certificates. A study of death certificates of all persons who died of cancer in the United States over an almost 20 year period, in addition to providing information on geographic clustering of certain forms of cancer, indicates a large increase in cancer mortality in the non-white population, over time. Negroes appear to be at particularly high risk of cancers of the respiratory system and the upper alimentary tract. The rate of increase of lung cancer among females is now greater than that for males. We should see a sharp increase in deaths from lung cancer among women over the next few years.

(3) Third National Cancer Survey. Data on the incidence of cancer in a 10% non-random sample of the population of the United States were published in a preliminary report in October 1971. These data will be followed by more complete incidence data covering a total of three consecutive years centered around the census year 1970. The major findings in the report were:

1. The rapid increase in cancer in Negro males.
2. The continued decrease in cancer incidence in women.
3. The continued increase in colon and rectum cancer incidence while mortality has remained nearly stable.

It now appears that the Survey should cover two years, rather than the three originally planned. The collection of data on the "rare" sites could continue for a third year.

The TNCS will provide a firm base for further work. For example, it will be possible to follow a sample of cases over a period of years to identify episodes of hospitalization, and establish events of medical and economic importance subsequent to diagnosis.

(4) End Results Program. The End Results data have pointed up the long survivals possible for patients with tumors showing no evidence of spread beyond the site of origin. Compared to persons of the same age without cancer, 20-year survival for this type of cancer patient is 85% for cancer of the uterus, 75% for ovary, and 60% for colon, rectum, breast, and bladder. A final publication will be issued late in 1972.

(5) Animal Cancers. Unusual aggregations of disease have been seen among domestic animals with malformations differing markedly among species. These observations are useful for both etiology and therapy studies, since the affected animals can be tested in ways that might be considered inappropriate

in man. In addition, the veterinary registers are identifying high risk groups that point toward similar studies in man.

(6) Industrial Epidemiology. The cancer risk to steel workers, smelter refinery workers, uranium miners, some white collar occupational groups, and 6500 U.S. Army World War II male radiology technicians has been examined. No form of cancer, including leukemia, was found in excess. In addition no genetic effects of radiation were discovered in their children.

In view of importance of the industrial environment as a possible source of cancer, the NCI will become more heavily involved in industrial epidemiology.

B. CHEMICAL CARCINOGENESIS. The observed relation between exposure to certain chemicals and the development of cancer in man has been historically the first step towards knowledge of the etiology of cancer. A listing of agents presently known to produce cancers in man includes some 10 specific chemicals, 12 mixtures and crude products and, in addition, radioactive materials and radiation (including x-rays and ultraviolet light). The number of items is relatively small because studies capable of identifying an agent as directly causative of cancer in man require large-scale and time consuming epidemiologic studies and have been conducted for a very small proportion of the potential exposures of man. In fact, essentially all materials that have been demonstrated to be carcinogenic for man have also been found to be carcinogenic in animals.

Most chemical carcinogens known from animal studies appear active in a variety of animal species. It is estimated that more than 1000 chemicals have so far been found carcinogenic in animal bioassays out of about 6000 that have been tested.

The major types of cancer in man that can be directly related to known carcinogenic exposures are as follows: (a) cancer of the lung related to cigarette smoke and certain other inhalation hazards (particularly asbestos, chromates, and radioactive materials); (b) cancer of the skin related to exposure to a variety of crude tar products and combustion products as well as to ultraviolet light and other forms of radiation; (c) cancer of the bladder related to exposure to aromatic amines and their derivatives; (d) leukemias related to exposure to radioactive materials; and (e) mesotheliomas related to asbestos exposure. A number of other human cancers have been considered to be related to exposure to environmental carcinogens but the evidence is less clear.

Because of the widespread use of some of these materials, the proportion of the total population exposed and affected is extremely difficult to estimate, with the exception of such materials as cyclamates, DDT and tobacco smoke. Since it has been estimated that the majority of the present cases of lung cancer in the United States are attributable to cigarette smoking, that factor alone appears to be responsible for a death toll in the neighborhood of 40,000 deaths a year. Current projects to produce a less hazardous cigarette must therefore be expanded.

The development of our knowledge on the causative factors of cancer in man can be projected along the following lines: (a) epidemiologic studies to correlate specific exposures to certain types of cancer in man with particular emphasis on occupational exposures; (b) additional bioassays in animal models which will identify chemicals as carcinogenic in animals and therefore potentially carcinogenic in man; (c) studies of animal human correlations by comparisons of bioassay results with the results of epidemiologic studies or by bioassays on isolated human tissues by *in vitro* techniques; and (d) by studies of pharmacologic and metabolic correlations among species.

The major areas of opportunity and need for progress in cancer prevention through studies in Chemical Carcinogenesis are: (a) develop better means for the removal of hazardous chemicals from the environment; (b) develop means to enhance the ability of the host to detoxify environmental agents; (c) determine the extent to which chemicals act in synergistic concert with viruses or with physical carcinogens such as irradiation; and (d) develop more rapid and more sensitive means to detect and bioassay the cancer-inducing effects of chemicals for man. The latter area of activity is particularly important. For example, it is estimated that approximately 200,000 new chemicals enter our environment each year. If only 500 to 2000 of these chemicals enter at reasonably high levels, and if only 10% of these are hazardous, then at least 50 to 200 new bioassays must be conducted per year. With present assays it takes approximately 2 years with 200 mice at approximately $200 per animal to test 1 chemical. It is hoped that newer studies in animal and human tissue cultures will diminish the 2 years and nearly $40,000 now required to test a single compound.

During the past year the following specific activities were begun or increased in emphasis:

(1) The Tobacco Working Group of the Lung Cancer Task Force was instituted with the mandate of identifying the criteria and prescribing the methods for the development of a less hazardous cigarette. Initially, the characteristics of tobacco and smoke were defined through the examination of agronomical and industrial practices, and the analytical mapping of chemical components. Simultaneously, a consensus was reached on the design of bioassay methods in laboratory animals, to monitor the carcinogenicity of smoke itself. Protocols for tobacco characterization, chemical analysis and bioassay have resulted. With the definition of updated experimental methods, the Tobacco Working Group devised an optimum experimental design for the development of a less hazardous cigarette, and recommended the testing of several cigarette types, representing modifications with a promise for a less hazardous product. Research and development contracts were awarded to implement the recommendations, with preliminary results expected in early 1972.

Current research includes (a) development of inhalation bioassay for small rodents, (b) testing of artificial tobacco substitutes (cellulose derivatives etc.), (c) testing of low nicotine-low wax tobacco varieties, (d) development of

drugs that may help discontinue the smoking habit (nicotine antagonists, nicotine antibodies, etc.), and (e) biometric surveys to determine real smoke intake doses in man.

(2) Bioassay. A new contract at the Stanford Research Institute, directed at establishing a ranking of suspicion of carcinogenic hazard for compounds present in man's environment, will allow the best utilization of current limited bioassay resources and may provide early guidelines to the chemical industry, as to the hazards that may be expected by the introduction of a new compound.

The significance of current bioassays for carcinogenicity derives mainly from two considerations, namely, that (a) humans in general react like other animals to a variety of insults, the differences being usually quantitative rather than qualitative and (b) current bioassay methods, even when performed with adequate biometrical and qualitative controls, can be expected to detect only those compounds that are strongly carcinogenic.

These two reasons prescribe that the greatest attention be given to positive results in current carcinogenicity tests.

Testing and retesting of environmental chemical pollutants at NCI continue to include agricultural chemicals such as pesticides, artificial sweeteners such as cyclamates and saccharin, hormonal compounds generally used as oral contraceptives, and natural products of various molds and fungi. Food preservatives are also under constant scrutiny. Recent findings suggest that human digestion and storage of fish and meat products preserved with $NaNO_2$ might produce carcinogenic nitrosamines. An investigation by S. Mirvish revealed that nitrosamine formation may occur in food stored under mildly acidic conditions. Two industrial chemicals widely used in consumer goods have been shown to be carcinogenic: propylene imine, produced in large quantities and used in a variety of solvents and adhesives, and propane sultone, produced in small quantities and used as a solvent in printing inks, dye pastes for printing yard goods, etc.

(3) Development of Bioassay Models. Among the more recent accomplishments are (a) a method for the induction of bronchogenic carcinomas by intratracheal instillation; (b) inhalation chamber techniques; (c) a method for induction of glandular stomach cancer by administrations of low-protein, high-salt diets with carcinogens; (d) induction of urinary bladder cancer in hamsters by feeding aromatic amines; (e) a model for the determination of host-mediated mutagenic effects of carcinogens in neurospora; and (f) development of *in vitro* studies of neoplastic transformation by chemicals.

(4) Identification of Carcinogenesis Processes. Studies on the microsomal enzyme have indicated that these are responsible for the hydroxylation and activation of polynuclear hydrocarbons carcinogens. Thus, an enzymatic basis for the covalent binding of polynuclear hydrocarbons to DNA has been discovered.

C. VIRAL ONCOLOGY. The probability is very high that viruses responsible for at least some human and domestic animal cancers have been found and that it will be possible to develop measures (including test vaccines) for the prevention or control of these diseases.

There are now over 100 viruses which are known to cause virtually all kinds of cancer in every major group of animals including sub-human primates. Many of these viruses will not only replicate, but will induce "cancerous" transformations in human cells grown in tissue cultures. The direct application of this laboratory know-how to human disease has revealed that virus particles of 3 types can be detected and in some cases isolated from patients afflicted with different types of cancer--Type C, Type B, and herpes Type (HTV). Particles of one kind (Type C) are identical with those known to induce leukemias and sarcomas in laboratory animals. Current studies by Huebner and others have culminated in one of the most exciting, unifying hypotheses of cancer etiology yet postulated.

A unifying hypothesis of cancer etiology. The highly predictable natural incidence and behavior of most cancer in animals as well as in man, plus a number of new discoveries and the resulting development of new concepts and insights, led some investigators to concentrate on the unique C-type RNA tumor virus genome as the most likely important viral cause of the generality of cancer. These new concepts and approaches, and the results of studies carried out in 1970 were epochal, since they led to a testable new hypothesis concerning the basic inherited nature of the RNA viral genome as a general cause of cancer and to a unitary theory capable of explaining spontaneous cancer as well as cancer evoked by exogenous environmental pollutants, endogenous physiological aberrations, genetic defects and mutations, as well as cancer clearly induced by viruses in experimental animals.

The hypothesis of Huebner and Todaro, et al. proposes that cells of probably all vertebrates have RNA tumors virus genomes of the C-type. These genomes are postulated to be transmitted from parent to offspring, and from cell to daughter cell, as part of normal inheritance. Host regulator genes and the repressors and various environmental carcinogens are regarded as factors controlling the expressions of the oncogene(s) and virogene(s) of the generally switched off viral genome. This hypothesis therefore views cancer as a natural biological event determined by spontaneous and/or induced "switch on" or derepression of universally prevalent specific viral oncogenes, thus providing a possible basis for a unifying theory of cancer which is consistent with naturally occurring cancers, as well as with those induced by radiation, chemicals and viral agents. Genetic defects, mutant genes, inducing agents, and finally the aging process itself, all appear to act to decrease the repression of the endogenous virogene(s) and oncogene(s).

Intrinsic enzymes of RNA "Tumor Viruses". A new series of findings which, among many other things, may contribute to the identification of a "switch" for the Huebner hypothesis are the detection and characterization of performed enzymes in RNA tumor viruses.

Genetic expression in bacterial cells is known to be mediated by transcription of the genetic information, coded in the form of base sequences in DNA, into RNA molecules followed by translation of the RNA into specific proteins. Until recently the operation of a similar mechanism in mammalian cells was unquestioned. In recent months Temin and Baltimore independently reported that RNA-containing oncogenic viruses contain a polymerase enzyme, which transcribes the genetic information contained in the RNA of these viruses directly into DNA. This finding, and others, have been fully confirmed and extended by Spiegelman et al. and Green et al. who have shown that the DNA formed is complementary to the viral RNA. More recent studies have revealed the presence of this enzyme in visna virus and in several simian foamy viruses, but not in approximately 10 other non-oncogenic RNA viruses of animal and tumor origin. Of further interest and apparent importance are reports by Spiegelman et al. and Gallo (NCI) that this polymerase is present in peripheral leukocytes from 5 leukemic patients but not in cells from 10 normal persons. These findings are important because this unique mode of replication of oncogenic viruses, through a RNA-DNA hybrid molecule, provides the possibility of selectively interfering with this step by chemotherapy, utilizing components which may interfere with DNA-RNA hybrid formation. Thus, new avenues for the chemotherapy control of viral produced cancer are opened. The polymerase enzyme can be utilized in the laboratory to prepare the DNA complement of the RNA-containing oncogenic viruses. This complementary DNA has been shown by Spiegelman to bind with high selectivity and form a DNA-RNA hybrid with the RNA of the oncogenic virus. Thus, the DNA made in the laboratory can be used for virus detection.

RNA Oncogenic Viruses in Man. For the past several years, sufficient numbers of observations reporting the finding of particules similar to the Type C and Type B in human malignancies have been made to conclude that viruses of these types may also infect man. The occasional presence of mature virus particles in human tumors, however, may be the exception rather than the rule.

With the development of new immunological and biochemical methods to detect Type C and Type B viruses or virus expression (polymerase activity assay, radioimmunoprecipitation inhibition assay, etc.), the finding that thymidine analogs (BrdU) are efficient virus inducers, and recent advances in molecular biology, the possibility of finding virus or virus expression in human tumors has increased by several orders of magnitude. Within the last year alone, four candidate RNA tumor viruses have been isolated and are being propagated in cell culture: ESP-1, RD-114, "Stewart virus", and a virus from the milk specimen of a woman with breast cancer. At the present time only two of these viruses are well enough characterized to describe further. ESP-1 was first isolated from cultures of cells in the pleural effusion from an American-type Burkitt's lymphoma patient. Investigations on the true origin of this virus are still under way and should be resolved soon. RD-114 virus was isolated from culture of a brain tumor of a fetal cat inoculated with human rhabdomyosarcoma cells. Intensive studies have already shown that the virus lacks the gs-1 antigen and shares the gs-3 antigen of known mammalian Type C viruses;

the polymerase is related to the enzymes of the recently isolated primate Type C viruses. More data are obviously needed to confirm its human origin. About a year ago evidence from electron microscopic studies was presented that human milk specimens, particularly those obtained from women whose family histories would indicate increased risk to breast cancer, contained particles similar to the known mouse mammary tumor virus. It was then shown that the presence of such particles coincided with the presence of detectable reverse transcriptase activity in human milk. The procedure was also used to demonstrate the presence of appropriate RNA and reverse transcriptase in purified viral particles isolated from human milk specimens, and to monitor and establish conditions for the successful infection and propagation of the suspected human breast cancer virus in human cell cultures. Preliminary results from these studies seem promising. Current Data also suggest that human breast tumors contain functional genes which are related to the genes contained in the virus known to induce mammary tumors in mice. Human leukemias and sarcomas also appear to contain different functional genes related to the viruses known to induce leukemias and sarcomas in mice and other animals. In each case, the viral-related genes in the human tumors are functional in that some proteins are presumably being synthesized. At this time, it is not known if all the proteins required for production of complete viruses are being synthesized or whether synthesis is restricted to include certain proteins which are involved in the cancer transformation of the cell. It can, however, be anticipated that efforts will be expanded in the coming year to determine the possible implication of both the virus particles obtained from human milk and the RD-114 virus in human cancers.

DNA Tumor Viruses. There is evidence linking the Etiology of several human tumors to DNA viruses, especially those of the herpesvirus group. As with RNA tumor viruses, diseases of animals have provided useful model systems. Herpesviruses recovered from wild rabbits, chickens, and higher primates induce lymphoproliferative neoplasias in their respective hosts and the association of a virus from frogs with kidney carcinoma is well known.

A herpesvirus, the Epstein-Barr virus (EBV), may be a contributing factor in the genesis of Burkitt's lymphoma. The mode of infection and site of production of EBV in this disease is unknown. A virus cause of this tumor is suspected because it occurs in high incidence in regions of Africa favorable for insect-vectored disease. Moreover, cases in certain areas cluster in time and space. EBV has been regularly isolated from tumor tissue, suggesting this virus may be the cause of the disease. Most patients with tumor have high titers of antibody to the viral antigens. However, a large proportion of normal people of all ages have antibodies to the virus, though usually in low titer. Further studies have shown that primary EBV infection of young adults caused some forms of infectious mononucleosis. Therefore, EBV may simply be a passenger isolated with regularity because of its predilection for the lymphocyte.

While there is good reason to believe that EBV is involved in the oncogenic process, its role could be that of an accessory factor. High antibody titers to virus antigens are consistently found in patients with diseases other than Burkitt's tumor. These include nasopharyngeal carcinoma, the sarcomatous form of Hodgkin's disease, and some cases of sarcoidosis. Although examples of a

causal relationship between one virus and different manifestations of malignant disease are known in avian and mammalian systems, the situation with regard to EBV is one of apparent rather then demonstrable direct association.

Genital strains of Herpesvirus hominis Type 2 might be a contributing factor in carcinoma of the uterine cervix. Seroepidemiological studies conducted in several cities in the United States showed that significantly more women with invasive carcinoma have antibodies to this virus than do women without this tumor. The incidence of this neoplasm is related to sexual experience. The cancer is rare in nuns and most prevalent in promiscuous women, where the exposure to infection by the virus is expected. When these studies were extended to selected groups of women in other countries, the results were not as conclusive. The question arises whether the methods applied are not sufficiently discriminating to permit detection of antibodies to certain genital strains in the presence of antibodies to oral strains of the virus, or whether the tumor-virus relationship is simply fortuitous. According to some investigators, there remains a significant difference between the rate of herpesvirus Type 2 antibodies in cervical patients compared to controls, and further epidemiological studies will continue in this area.

BREAST CANCER STUDIES

Until recently, research activities in breast cancer have been limited to exploratory studies in search of evidence that viruses might be associated with human breast cancer. The consistent finding by electron microscopy of particles resembling the known RNA tumor viruses in human materials, and the successful isolation and propagation of three candidate viruses -- one of subhuman primate (Rhesus monkey) and two of human origin -- have led to the theory that viruses of this type should be considered as major oncogenes for animals "higher" than the mouse.

Isolation of a Virus From a Monkey Breast Cancer. The most advanced and one of the most significant developments in breast cancer is represented by the discovery, isolation, and successful propagation of a C type virus associated with an adenocarcinoma of the breast of a Rhesus monkey. It has been designated as the Mason-Pfizer Monkey Virus (M-PMV). This is the first C type virus of any primate thus far propagated in sufficient quantities to support systematic investigations on its virological, biological, immunological, chemical and physical characterization. The M-PM virus grows readily in primary Rhesus monkey embryo and fetal lung cultures, and to a lesser extent in cell lines of chimpanzee lung, human embryo and human lymphoblasts (NC-37).

> Antigens of the Mason-Pfizer monkey virus have been found to be present in two of five normal Rhesus monkey embryos. This finding suggests that M-PMV may be indigenous and vertically transmitted in this species.

The M-PM virus was shown by Chopra, et al. (NCI) to form from precursor intracytoplasmic A Type particles which bud through the cell membrane as in the formation of B particles of mouse mammary tumor virus, but not to have spikes characteristic of the latter. The mature particle is indistinguishable from

C type particles in ultrastructural morphology although the nucleoid does not form during budding, at cell membranes, as do all of the other known C type viruses. The M-PM virus therefore possesses certain characteristics of both B and C type viruses. The nucleic acid is of the RNA type and the virus particles contain RNA-dependent DNA polymerase as do all known oncogenic RNA viruses thus far studied (Spiegelman).

Demonstration of an RNA virus in Cell Lines Derived from Human Breast Cancer. Two groups of scientists have demonstrated morphologically similar RNA viruses in tissue culture lines derived from two different cases of human breast cancer. The former investigators also reported isolating the same type of virus from two cases of cancer of other types, among a total of 56 specimens from human cancer patients studied.

The virus produces no CPE or other morphological evidence of its presence, but it can be detected both by ^{3}H-uridine incorporation and by electron microscopy. The virus is found in appreciable amounts in tissue culture fluids, and can infect other human cell lines. It resembles the mouse mammary tumor virus (MTV) in that it has peripheral spikes of identical size, spacing and morphology but it is only 2/3 the size of the MTV, i.e., 50-75 mu as compared with 100/140 mu. Except for its slightly smaller size, therefore, it resembles the only presently known breast cancer virus (MTV).

Moore and associates have reported finding particles resembling the B type virus of mouse mammary cancer in 5/75 (6.7%) milk specimens from normal women in the Philadelphia area. In further studies as yet unpublished, much higher frequencies of similar particles have been found in milk from women of special or high risk population groups.

No human candidate breast cancer virus is yet available in sufficient quantities to support seroepidemiological studies, but attempts to scale up production of one of them are underway. The same bank of specimens used in the M-P monkey virus tests are on hand to be tested similarly with the human agents as they become available.

DISCUSSION

Between 30% to 90% of cancer deaths in man is preventable. Mortality statistics from around the world show that if cancer mortality in the United States, site for site was comparable with that of the lowest country reporting for each site, approximately 100,000 lives would be saved each year. The major sites in which gains should be made are cancer of the colon and rectum, cancer of the lung, breast cancer, cervix and uterus (particularly among non-whites), cancer of the ovary, cancer of the prostate, and probably leukemia.

Information is already in hand to reach these goals for lung cancer and for cancer of the cervix and uterus. These 2 sites alone would account for 60,000 or more savable lives. A concerted research effort is called for in cancer of the colon, breast cancer and cancer of the prostate. There is promise of substantial pay-off in these sites. For example, migrant studies have provided very strong evidence that cancer of the colon is a disease with a very strong

environmental base. Japanese migrants to Hawaii show a 3 to 5-fold increase in contrast to Japanese on the home islands. When we find those things in the new environment that lead to so large an increase, application of this knowledge to the present United States population could reduce the U.S. incidence by another 25-30,000 lives a year.

Thirty percent (or 100,000 lives) is a minimum estimate. If we add to the preventable cases (in terms of current knowledge, or knowledge that should be available in the next few years, earlier diagnosis and improved treatments--where prevention has broken down), this number should be easily doubled. For example, if the most recent results of the HIP-NCI (Health Insurance Plan-National Cancer Institute, unpublished) studies on early diagnosis of breast cancer were applied across the country, we might anticipate 12,000 fewer deaths each year from breast cancer alone. If the finding that early pregnancy (before age 20) cuts in half the probability of developing breast cancer, could be translated into a prophylactic treatment for young women (i.e., create the hormonal milieu of pregnancy without actual pregnancy) another substantial reduction in breast cancer deaths would follow.

Current work on identifying the cancer "susceptibles", as successful, will lead to cutting down cancer deaths 2 ways. First, by identifying susceptibles, we will know what people to watch closely and regularly, so that we might diagnose their disease early, and thus treat it successfully. Second, by identifying the cancer susceptibles, it should be possible to protect them from those environmental assaults which could be carcinogenic to them--while essentially innocuous to others.

Ninety percent reduction in cancer deaths could be reached within the present century, if, in addition to current knowledge on prevention, and universal application of the best diagnosis and treatment techniques, our research uncovered (a) the environmental elements that cause migrants' cancer rates to change toward those of their new countries; (b) those environmental elements which lead to different rates in different countries in the world; and (c) the susceptibles whom we must and can protect.

While there are major problems in the prevention of virus-induced cancers, not heretofore experienced in other areas of infectious disease, we should anticipate substantial inevitable success in the control (including prevention) of these diseases in man. These problems include the probable difficulties in using conventional vaccines for prevention if a major means of virus exposure is vertical rather than horizontal. Secondly, the "peaking" phenomenon of acute lymphocytic leukemia in Caucasian children at about 4 years of age and other similar phenomena suggest that it would take 5-10 years before the results of a preventative field trial were known. Even with a "clustering" disease of relative high incidence, such as Burkitt lymphoma in equatorial Africa, nearly 1,000,000 children would have to be included in the "treated" cohort observed for 5-8 years to determine whether the expected 40-50 lymphomas would be eliminated or decreased.

These difficulties notwithstanding, opportunities for cancer prevention and control are very bright through application of existing knowledge and through a prioritied commitment to seek new information with innovative application for man.

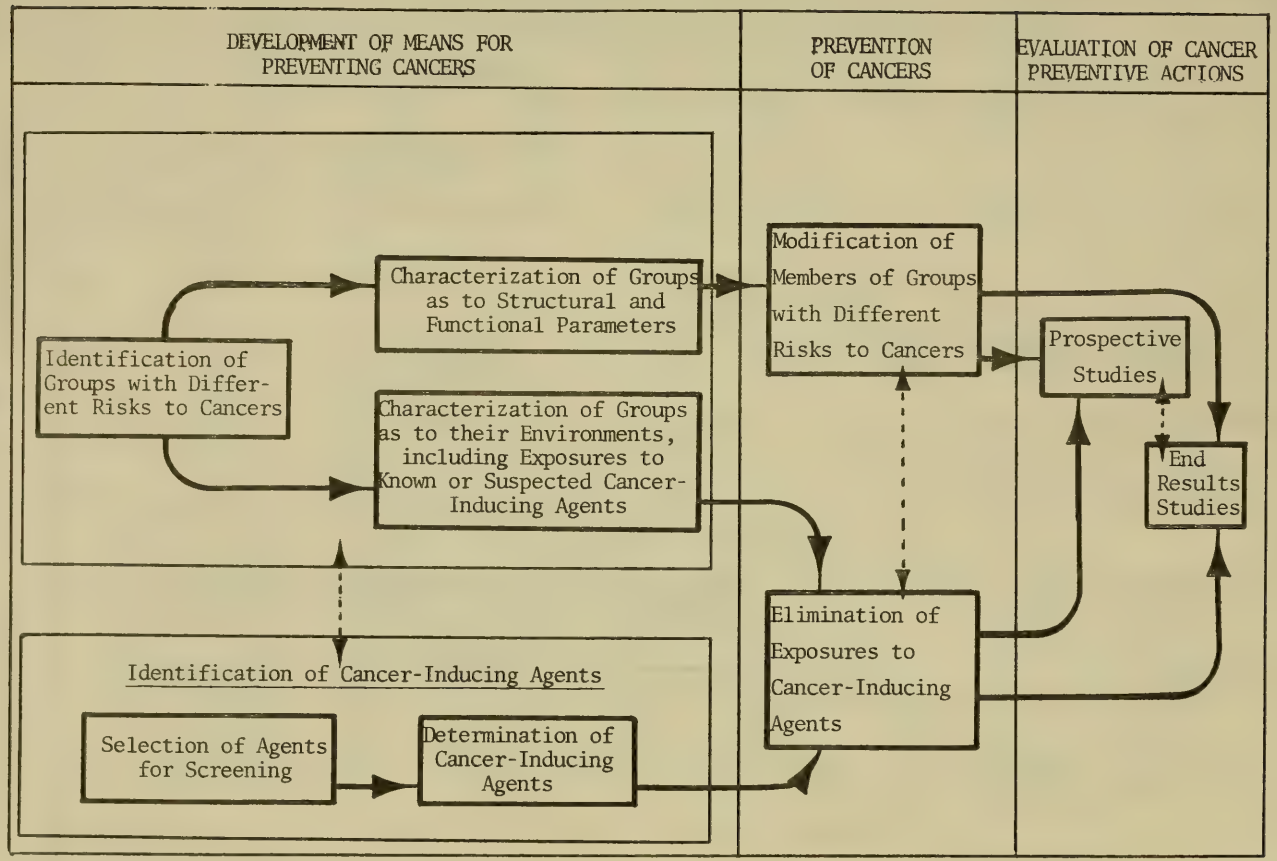
A Note on Management. The obligatory opportunity to plan and manage biomedical activities from basic or fundamental research upwards through development and application, with effectiveness and efficiency, requires skillful staff and line personnel of unabashed motivation, objectivity, compassion and tact. Tact is of paramount importance because NCI management must skillfully and with objective credibility persuade constituent scientists to attack specific problems of human disease. We adhere to the belief that NIH exists primarily to allow people to live better and longer and that NCI abets this goal through the conduct of research and development for prevention and treatment of cancer in man. Tact, including the courage and reason to say no, is important also because while the need for widespread program participation is high, many scientists do not understand that even successful completion of their proposed project may not necessarily contribute to the attainment of program goals. This thrust towards the timely control of disease must be tempered with appreciation for and selection of fundamental projects with some expectation of contribution to eventual application to man. This can and should be do through both grants and contracts.

We are fortunate to have this admixture of talents and motivation in the Associate Scientific Directors, Drs. Gori, Moloney, Saffiotti and Schneiderman; in the Deputy Scientific Director, Dr. Peters; the Assistants to the Scientific Director, Drs. Bryan and Depue, and in the Administrative Officer, Mr. John Patterson. Particular recognition is entirely warranted to their associates whose motivation to program goals clearly exceeds desire for personal recognition.

Please refer to summary and individual reports for details of activities within Etiology programs for FY 71.

CANCER ETIOLOGY AND PREVENTION PROGRAM

FIGURE 1


DEVELOPMENT OF MEANS FOR PREVENTING CANCERS
PREVENTION OF CANCERS
EVALUATION OF CANCER PREVENTIVE ACTIONS
Identification of Groups with Different Risks to Cancers
Characterization of Groups as to Structural and Functional Parameters
Characterization of Groups as to their Environments, including Exposures to Known or Suspected Cancer-Inducing Agents
Identification of Cancer-Inducing Agents
Selection of Agents for Screening
Determination of Cancer-Inducing Agents
Modification of Members of Groups with Different Risks to Cancers
Elimination of Exposures to Cancer-Inducing Agents
Prospective Studies
End Results Studies

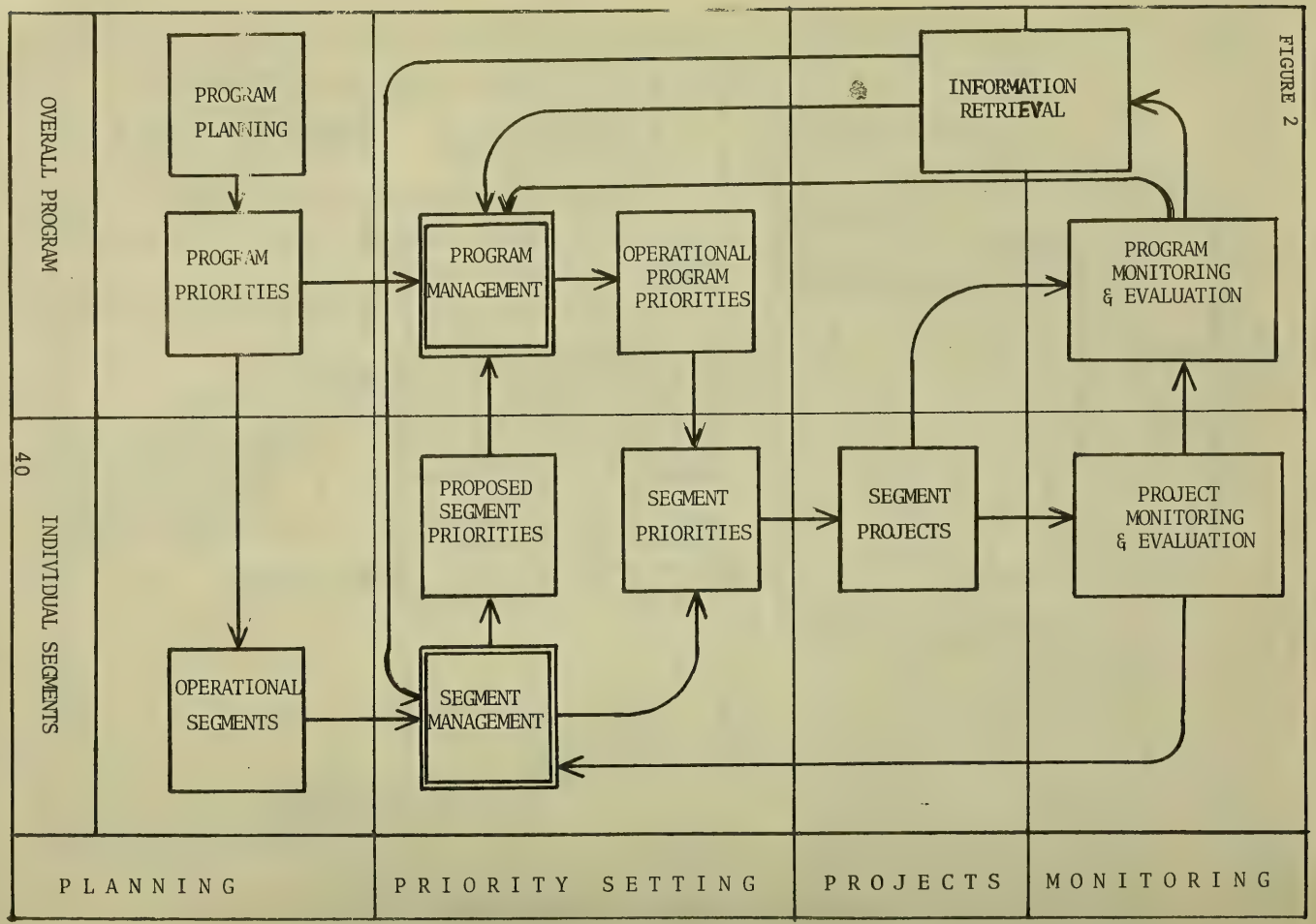

OVERALL PROGRAM
INDIVIDUAL SEGMENTS
PROGRAM PLANNING
PROGRAM PRIORITIES
OPERATIONAL SEGMENTS
PROGRAM MANAGEMENT
OPERATIONAL PROGRAM PRIORITIES
PROPOSED SEGMENT PRIORITIES
SEGMENT PRIORITIES
SEGMENT MANAGEMENT
INFORMATION RETRIEVAL
SEGMENT PROJECTS
PROGRAM MONITORING & EVALUATION
PROJECT MONITORING & EVALUATION
PLANNING
PRIORITY SETTING
PROJECTS
MONITORING

FIGURE 2

TABLE 1: CANCER INCIDENCE: EXPECTED NUMBERS, 1970-2000; CHANGE IN RATES, 1970-2000; DEATHS, 1970

SITE	NEW CASES (INCIDENCE) 1970	2000	DEATHS	INCIDENCE YEAR 2000 % CHANGE* IN RATE	MAJOR CAUSATION	MEANS OF PREVENTION
Lung	63,000	144,000	62,000	52	Tobacco Smoke Air Pollution (including on-the-job)	Stop smoking - Reduce pollution Less hazardous cigarette
Large and Small Bowel	75,000	143,000	44,000	40	Intestinal Flora? Heredity? Diet?	Viruses? Other insults. Identify susceptibles and eliminate their exposure.
Breast	69,000	121,000	30,000	25	Virus? Diet? (Hormones) Genetic?	Vaccines. Identify susceptibles.
Pancreas	19,000	101,000	18,000	180	Diet? Virus? Other Insults?	Identify etiology. Identify susceptibles.
Prostate	35,000	61,000	17,000	27	Hormones? Diet?	Identify etiology. Identify susceptibles.
Stomach	17,000	5,000	16,000	(80) decrease	Diet Poor socio-economic conditions	Diet modifications. Sociologic modifications?
Leukemias	19,000	33,000	15,000	25	Viruses. Radiation. Genetic.	Vaccines. Identify susceptibles and limit radiation.
Skin	112,000	186,000	5,000	20	Actinic rays. Genetic.	Limit radiation exposure. Identify susceptibles.
Misc.	120,000	217,000	75,000	30	Multiple	Identify extrinsic and intrinsic factors and modify them.

*Expected change in age adjusted incidence rates. Year 2000 compared to 1970.

OFFICE OF THE ASSOCIATE SCIENTIFIC
DIRECTOR FOR PROGRAM

Contract Narrative

WOLF RESEARCH AND DEVELOPMENT CORPORATION (NIH-71-2270)

Title: Develop and Implement a Program Management System (PMS)

Contractor's Project Director: Dr. William Lake

Project Officer (NCI): Dr. Gio Gori

Objectives: To (1) analyze the requirements for collaborative program management information at all levels of Etiology; (2) design a system to provide the information; and (3) implement and operate the system.

Major Findings: (1) During the first year the analysis of information needs covered the functional areas of Program, Contracting and Administration. Organizationally the study concentrated initially on the Carcinogenesis Area, then was expanded to include coverage of Viral Oncology and Demography to insure the development of a fully articulated system. The generation of information, its flow and use were documented in a series of interim reports. (2) A broad system design, including manual and computer functions and man-machine interfaces was developed. A flexible data-base was designed, using the contract-task as the structural unit. (3) Initially, data was collected and processed to produce pro-forma output reports for several levels of management in the major functional areas. This prototype operation provided the necessary user feedback to iteratively improve the system design. The total system was then "frozen" in order to proceed to full implementation. The PMS is being brought on-stream first in the Carcinogenesis Area, and will be successively expanded to serve Viral Oncology and Demography.

Significance to Biomedical Research and the Program of the Institute: The management of an expanding collaborative research program demands a flexible and responsive system to provide science managers timely and accurate information. The PMS is designed to provide this needed information system.

Proposed Course: Continuation of the phased implementation, into full, Etiology-wide operation. Evaluation of PMS operations, and system enhancement to meet identified user requirements.

Date Contract Initiated: May 3, 1971

Current Funding Level: $104,000

A. SUMMARY REPORT
OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR
FOR DEMOGRAPHY, ETIOLOGY
JULY 1, 1971 - JUNE 30, 1972

The Demography Area of Etiology has three general purposes:

1. To uncover the relationship of man's environment {internal / external} to the development of cancer for three purposes:

   (a) Find ways to prevent cancer by finding "causes" that can be manipulated.
   (b) Find ways to diagnose early (and thus treat better).
   (c) To help develop and evaluate theories of cancer etiology.

2. Develop and provide mathematical, statistical, and computational-organizational techniques to enable research workers in other areas to proceed more rapidly and more validly toward the solution of their research problems (in cancer and related fields) and to evaluate the effectiveness of programs in diagnosis, treatment and cancer control.

3. Provide basic information from which the magnitude of the cancer problem (as a public health problem) can be measured and from which it will be possible to develop sound policies on the delivery of health care, on the modification and protection of the environment (e.g. pollution control) and other activities to protect the population of the nation from developing cancer.

In the past year substantial work has been done in relation to all three purposes:

1. (a) Studies in children's cancer of various types have shown relationships between congenital abnormalities and cancer. This work is being extended through cooperation with the UICC and with the National Institute for Environmental Health Sciences in a manner that will encourage the investigation of relationships by many workers, thus increasing the probability of uncovering things of importance.

   (b) Clues from earlier migrant studies have led to investigations (in Hawaii and Japan) of the role of bacterial flora, and of adenomatous polyps in colon cancer.

   (c) Substantial racial differences have appeared in the changing incidence and mortality from cancer. American Indian men have lower than expected mortality from cancer of the lung, stomach, pancreas, colon and prostate. American Indian women have low breast cancer rates, but high rates for liver, pancreas and uterine cervix. The sex difference in pancreas

cancer needs explanation. Negro men now have the highest cancer incidence rates in the United States with particularly high rates in lung, esophagus and prostate.

(d) Drugs appear to be less important causes of cancer in man than chemicals to which people are occupationally exposed. Nonetheless, investigations of the long-term effects of Hycanthone (used for treating schistosomiasis) are under way. A study of Atabrine, a drug related to Hycanthone, in WW II veterans showed no unusual patterns of cancer mortality. By examining mortality data for the counties on the western slope of the Rocky Mountains in Colorado, it has not been possible to confirm a postulated increase in cancer in families living in houses surrounded by radioactive uranium mine tailings.

(e) Several attempts have been made to test, in man, the various hypothesis relating to a viral etiology of cancer. To date, the epidemiologic data do not support this hypothesis in breast cancer, and two studies relating infectious mononucleosis and Hodgkin's disease have produced contradictory results. We have not been able to independently verify time-space clustering in Hodgkin's disease. Consultation is continuing in support of studies of the possible relation of EB virus, Burkitt's lymphoma and nasopharyngeal carcinoma. A two-stage mechanism involving malaria infection may be needed to explain the EBV-Burkitt relationship.

(f) Studies of populations developing colon cancer, with particular emphasis on migrants, lead to the hypothesis that there is a decisive etiologic role for some vents which take place before age 15. Then some events in adult life lead to the expression of disease. This model, and the data on which it is based, is consistent with the initiator-promoter model of animal carcinogenesis put forward by Berenblum.

2. Extensive consultation has been given the scientific staffs at NCI, in particular with respect to data analysis and interpretation of the experiments designed to develop a safer cigarette, to the conduct of large scale controlled clinical trials under conditions of greatly restricted randomization, and in the development of mathematical models of the carcinogenic potential involved in the interaction of two or more materials.

   (a) Mathematics of histo-compatability and HLA typing have been developed. Problems of tissue transplantation and individual susceptibility to cancer appear to be related in that similar mathematics are applicable to them.

(b) Consultation has been given to the Food and Drug Administration with respect to general problems of safety testing, and the specific problems posed by the use of Diethylstilbestrol in animal feeds, and NTA in detergents.

(c) Consultation in staging systems has been given to the American College of Surgeons with respect to several diseases and to several other Federal agencies, WHO, and IARC on a wide variety of problems.

3. (a) Data on the incidence of cancer in a 10% non-random sample of the population of the United States were published in a preliminary report in October 1971. These data will be followed by more complete incidence data covering a total of three consecutive years centered around the census year 1970. The major findings in the report were:

1. The rapid increase in cancer in Negro males.
2. The continued decrease in cancer incidence in women.
3. The continued increase in colon and rectum cancer incidence while mortality has remained nearly stable.

(b) The End Results program has assembled data on treatment and survival beginning in 1940. A full publication of these data will be available at the time of the National Cancer Congress in September-October 1972. The major findings that will be reported are:

1. The rather small improvements in early diagnosis that have occurred for most sites. Sites of most improvement are:

	Per cent Diagnosed Localized	
	1940-49	1965-69
Breast	38	47
Prostate	49	63
Bladder	67	79
Melanoma	52	73
Brain	71	86

2. Sites showing important improvement in survival are:

	3-year Relative Survival Rate (%)	
	1940-49	1965-69
Acute lymphocytic leukemia	0	15
Larynx	47	67
Prostate	49	66
Bladder	48	62
Melanoma	49	74
Brain	28	37
Thyroid	67	86
Hodgkin's disease	35	61
Multiple myeloma	10	27
Chronic lymphocytic leukemia	33	53
CML	16	26

3. Major improvements in survival have not been noted in:

	3-year Relative Survival Rate (%)	
	1940-49	1965-69
Esophagus	2	5
Stomach	12	15
Liver	4	6
Pancreas	2	2
Lung & Bronchus	6	11
Ovary	30	35
Pharynx	25	32
Reticulum cell sarcoma	22	22
Lymphosarcoma	33	40
AML	3	2

(c) Data have been made available of 8634 tumors among 202,277 animals seen through 1969 which have revealed several items of consequence to man:

1. High risk groups have been uncovered -- indicating some genetic predisposition.
2. A substantial sex-difference (4-fold) has been noted in a non-sex organ cancer (orbit of the eye) in cattle.
3. Four different types of congenital defects in dogs have been uncovered which may provide some excellent animal models for studying similar defects in man.

Plans and Future Work

Following the recommendations of an Ad Hoc Advisory Committee on Population-based Registries, the Biometry Branch has begun developing cancer registries with an epidemiologic and etiologic orientation in several areas of the country. These include Massachusetts, New Mexico, Hawaii, and part of California. An important part of the work in these areas will be to develop adequate cancer epidemiologists and statisticians who are in very short supply.

Data will be developed from the Third National Cancer Survey on costs of cancer, patterns of referral and treatment and other items useful for planning and evaluating cancer control programs.

Prognostic factors in other diseases in addition to breast cancer will be developed with early emphasis on Hodgkin's disease.

A minor reorganization of the Biometry Branch will create a Clinical Biometry Section concerned with consultation on the conduct of clinical trials, and with the review of end results data to indicate where further research in therapy should prove most useful.

Extended work will be done in the statistical theory of diagnostic tests, with application to the evaluation of candidate diagnostic tests by other areas of NCI.

Involvement with the epidemiology program of the Atomic Bomb Casualty Commission will provide extensive data which will permit testing various hypothesis of the etiology of cancer, particularly with respect to radiation. The involvement with ABCC will lead to the training of more cancer epidemiologists-people now in very short supply.

The "alert practitioner" program which attempts to increase the awareness of the bedside physician to possible environmental causes of disease will be extended through cooperation with the American Academy of Pediatrics. This program should provide leads to environmental causes of disease, and a group of physicians who are able to think etiologically, and thus move toward disease prevention.

Problems

The major problems in the Demography Area derive from three sources:

1. The very wide scope of interests covered by this misnamed area (demographic research is only one facet of the program).

2. The demands on the staff for involvement in all the fields in which there is reason or need to be involved, either as primary research workers, or as consultants, or as project officers on contracts.

3. The small number of trained cancer epidemiologists and statisticians that can be called upon to do the necessary work.

Expected increases in staff will help. Developing skills to fill the vacant positions will be harder. Finding knowledgeable people to serve as project officers for projects carried out elsewhere will be hardest of all. When demands begin to be made in the next several years for the evaluation of cancer control programs, and to weigh the effectiveness of "out-reach" programs of the Comprehensive Clinical Cancer Research Centers, it is quite likely that there will be too few trained people to meet the needs, despite our attempts to increase training in many of our programs.

CONTRACT NARRATIVE
OFFICE OF THE ASSOCIATE SCIENTIFIC
DIRECTOR FOR DEMOGRAPHY, ETIOLOGY

Fiscal Year 1972

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO (NIH-E-71-2180)

Title: Group Testing for Screening Carcinogens

Contractor's Project Director : Dr. Robert M. Elashoff

Project Officer (NCI): Dr. Marvin A. Schneiderman

Objectives: To develop mathematical techniques based on the theory of group testing to permit more rapid and efficient testing of chemicals for potential carcinogenesis.

Major Findings: Maximum tolerated dose schemes have been developed for a pilot study in which mixtures of materials are administered to each test animal, rather than a single compound to each animal. These designs are constructed to facilitate development of mathematical models of interactions in carcinogenesis.

Two mathematical-statistical papers were prepared for publication -- one on Estimation by Pooling, and another on Applications of Group Testing to Cancer Diagnosis.

Significance to Biomedical Research and the Program of the Institute: Carcinogenesis testing in vivo is a long-term expensive process. Current testing methods have provided no general theories of interaction of materials, and in the real world persons are always exposed to many materials rather than one. This research should serve to do two things:

1. Speed up testing of materials.
2. Develop models of interaction of materials, so that the effects of multiple exposures might be better understood and perhaps become amenable to forecasting.

Proposed Course: Continued close collaboration with groups doing biological testing of the materials to speed the development of mathematical models.

Development of non-sequential group testing schemes (a compromise between financial economy and time-economy).

Development of further applications of group testing.

Date Contract Initiated: June 24, 1971

Current Annual Level: $35,380

B. SUMMARY REPORT
BIOMETRY BRANCH
July 1, 1971 - June 30, 1972

The Biometry Branch has three main functions -- to conduct research on the etiology of cancer in man; to provide statistical consultation and support for other people conducting research; and to provide baseline data against which administrators and researchers can measure their successes (and failures) in reducing cancer incidence and mortality. These functions encompass a wide range of scientific and managerial skills in bringing people and data together.

Third National Cancer Survey

The objective of the Third National Cancer Survey is to register all new cancer cases diagnosed in the resident population of seven metropolitan areas, two states, and the Commonwealth of Puerto Rico during the period 1969-1971; to obtain demographic data and a description of the tumor for each patient; and to study certain economic, social, and other aspects of the disease in a sample of patients.

A preliminary report based on the 1969 incidence data was distributed to the National Cancer Advisory Council in October 1971. The report showed that 61,410 cancers were diagnosed in the survey areas during 1969. Since the survey areas include ten percent of the total United States population, it is estimated that approximately 610,000 new cancers were diagnosed in that year. When the data are adjusted to the age distribution of the United States in 1970, the incidence rate for the black population (338 per 100,000) is substantially higher than for the white population (311 per 100,000). The sites with markedly higher rates among black males are the prostate and esophagus. Among females, the black females have higher rates for both invasive and in situ carcinoma of the cervix uteri while the white females had higher rates for cancer of the breast and corpus uteri.

While the areas covered by the present survey do not correspond exactly to the areas covered in the survey of 1947, some trends can be discerned. The age-adjusted incidence rates among males increased from 280 in 1947 to 304 in 1969 while the rates among females decreased from 294 to 256 (excluding carcinoma of the cervix uteri) for the same interval. The increase in the male rate is due largely to substanial increase in the incidence of cancers of the lung and prostate and to a somewhat lesser increase in colon cancer. These increases more than counterbalanced the declines for gastric and rectal cancers. The decreased female rate results from a drop in the incidence of cancers of the cervix uteri (invasive), stomach, and rectum.

Registration of all cancer cases in a defined population is the only way to acquire reliable data on cancer incidence. The data bank also provides a valuable resource for epidemiological investigations by identifying population groups with high or low incidence of specific sites.

Other products of the survey that will be important to the mission of the National Cancer Institute will be studies on the delivery of medical care to a large and representative sample of cancer patients, the treatment provided them, extent of the disease at the time of diagnosis, the extent and duration of disability from cancer, and direct and indirect costs of cancer.

Population-based Epidemiology Research Centers

The acceptance of the conquest of cancer as a national goal has placed increased emphasis on all aspects of the cancer problem including both research and action directed towards prevention, diagnosis, and treatment. Implementation of the many objectives encompassed in the national goal will demand, and to a great extent depend upon up-to-date and valid information to define the nature and magnitude of the total cancer problem and its components, and to measure progress in the areas of prevention, diagnosis and therapy. Mortality data alone are often inadequate measures of changes in incidence of cancer and of progress in the prevention of the disease. While the Third National Cancer Survey will provide incidence figures for the period 1969-71, there is as yet no provision for collecting data on a continuing basis to augment the limited information available from the Connecticut Tumor Registry, so that changes in incidence patterns can be monitored as they occur. The limitations of the inferences that can be derived from mortality data in no way reduce the critical need for a national death index. The latter has many specific research purposes and is fundamental to the design and assessment of programs to identify and abolish causes of cancer and to evaluate the effect of therapeutic intervention.

An ad hoc Advisory Committee on Population-based Epidemiology Research Centers met on November 8, 1971 and made several recommendations on this subject some of which are quoted below.

> "The Associate Scientific Director for Demography should develop a coordinated program of cancer surveillance, descriptive epidemiology and end results evaluation....
>
> "The current End Results Program should be used as one basis for developing the new program, while continuing and increasing its role in evaluating the long term results of treatment....
>
> "The new program should include an optimum mixture of participating groups....:

a) registries in individual cancer specialty centers and other university affiliated treatment centers,

b) population-based cancer registries,

c) comprehensive clinical cancer research centers as defined in the National Cancer Act of 1971,

d) population-based cancer study centers.

"The extramural grants program should be encouraged to capitalize on intramural program activities and to foster epidemiology and biometry training programs."

These recommendations are being implemented in Branch program planning. Plans for development of population-based registries in Massachusetts, Hawaii and New Mexico are progressing well. The Hawaii registry will hopefully become a part of a future comprehensive clinical cancer research center. The registries in California and Connecticut are scheduled for development into population-based cancer study centers. A basic requirement of these new registries is that they develop a sound incidence reporting system and a training program. Additional registries will be developed in other areas of the country as the program progresses. Collaborative efforts are being considered with registries in Canada and Israel.

End Results Program

The registries making up the End Results Group represent the major U.S. source of data on end-results of cancer treatment. While the program as presently constituted has yielded several important and significant findings, sustained work with the registry materials has indicated the need for more population-based registry data.

The End Results Section, in publications describing the survival experience of cancer patients, continues to satisfy numerous national demands for this type of information. A major comprehensive summary, "The End Results in Cancer, Report No. 4" has been completed and will be distributed at the Seventh National Cancer Conference to be held this year. This report provides the most comprehensive set of data now available on the diagnosis and treatment of cancer and the associated survival of patients.

Clinical Biometry

In the coming year we plan to organize a new section devoted to problems in clinical biometry. This section will be formed about an already existing nucleus of people responsible for the

statistical operations of the clinical trials conducted by the Veterans Administration Cooperative Urological Research Group. The new Clinical Biometry Section will be concerned with protocol design, data recording, collection and editing, and methods of routine and special analysis of data from prospective randomized clinical trails of cancer therapies. Emphasis will be given to descriptions of natural history of untreated patients. This Section may also act as a statistical office providing the above services for several large-scale clinical trials conducted by outside groups, and plans to provide consultation concerning operation and analysis of clinical trials, clinical decision-making and related problems to clinicians and scientists in the National Cancer Institute and other institutions.

Migrant Studies

The Branch studies of migrant populations oriented to change in site-specific cancer risks supported primarily by research contracts with investigators in the countries of origin, has continued to focus on migrants from Japan, United Kingdom, and Norway to the United States, and on intra-country migration in Colombia. The possibility that cancer in man may reflect an initiator-promoter process has been strengthened by the collective observations. One may hypothesize that "initiator" may involve events occuring very early in a person's life, so that unless the initial stage occurs in early life, cancer will not develop in later life, even though the promoting agent is present. The importance of early exposures varies with site. The recent sharp rise in colon cancer among Japanese migrants to Hawaii suggests that exposures occurring in adult life after arrival in Hawaii have modified the risks for this site.

Several migrant population studies have consistently delineated the great difference between stomach and colon cancer in the behavior of the risks in these populations. For stomach, migrants to the United States have consistently displayed risks more characteristic of the country of origin, suggesting a decisive etiological role for events in the first 15 years of life. The rise in colon cancer risks among migrants to approach the prevailing U.S. level suggests that events in adult life play a role, one possibility being that expression of a precursor state may be delayed to older ages. One candidate precursor lesion that would fit this pattern, adenomatous polyps, has been proposed. Adenomatous polyps tend to appear rather late in life and the proportion of polyp bearers under age 35 or 40 is small. This consideration, in turn, has introduced pathology into the study program. Comparative population studies on the congruence of colon cancer and adenomatous polyps have been initiated and such efforts will be extended to the Japanese migrant study. Colons from autopsies in Hawaii and Japan are being assembled in an effort to collate polyp findings with incidence data.

The point which emerges with increasing clarity is that the etiology of colon cancer is unlikely to be resolved successfully by studies conducted within the confines of a single population. Only with the broad perspective afforded by comparative population investigations has it been possible to distill ideas on etiology. Colon is probably not unique in this respect and the suspicion is growing that epidemiological findings within any single population will reveal few secrets and that clarification of the epidemiology of many new cancer sites will require the broad picture provided by comparative population studies of sedentes and migrants.

Japan-Hawaii Cancer Study

An outgrowth of the Japanese migrant study program is the Japan-Hawaii Cancer Study which utilizes the cohort of Japanese males in Hawaii assembled by the National Heart and Lung Institute for studies of cardiovascular diseases. Plans to expand the cohort to include wives and sibs of present members are envisioned.

The study includes extensive clinical and pathological research on the cohort population with emphasis on stomach, colon/rectum, thyroid, breast and prostate cancers. A special effort is being made to explore the role of diet and intestinal flora in the etiology of colon cancer.

Consulting

The Branch extended its consulting activities to many new cancer-related problems arising in research conducted at the National Cancer Institute. The staff of the Mathematical Statistics and Applied Mathematics Section during the past year worked closely with over a dozen branches and laboratories, ranging from problems in histo-compatability testing to the design and analysis of controlled clinical trials conducted under non-standard conditions. The Office of the Chief has provided consultation in problems of epidemiology, carcinogenesis, drug safety evaluation, problems of determining safety levels of radiation, problems of tissue transplantation, and the evaluation of new therapies, much of which have arisen in the course of contract-supported research. Extensive cooperation and consultation with the Food and Drug Administration, the National Academy of Sciences, the Atomic Energy Commission, the Federal Aviation Administration, and the Veterans Administration as well as the World Health Organization, and the International Agency for Research on Cancer has continued. Consultation has also been given to the American College of Surgeons, and the American Cancer Society.

Problems

The dimensions of the problems confronting the Branch set forth in preceding annual reports remain unchanged. The major constraint is the limited number of positions. The Third National

Cancer Survey has been slowed down by the tight personnel ceilings and this factor is forcing modifications in survey plans and some curtailment of the ambitious program as originally conceived. We are currently unable to entertain development of studies of employee populations greatly desired by investigators concerned with occupational carcinogenesis. In many respects the program of migrant population studies is a holding operation and only a small fraction of the lines of work that have been indicated can be pursued with present staff. The imbalance between available research contract funds and the size of the intramural staff may deteriorate, given larger appropriations for cancer research. The situation can be ameliorated in part by development of the prime contractor concept and a search for prime contractors to operate population-based cancer study centers which are of interest to the Etiology Area.

The integration of information yielded by clinical trials (a subject for which the Branch provides some supporting staff services) with end-results data provided by cancer registries needs to be improved. Reorganization of the Branch to reestablish a Clinical Biometry Section concerned with not only consultation on conduct of clinical trials but also with review of the end-results data to define topics amenable to further investigation by clinical trials should improve matters.

Despite these caveats the future prospects look good. Mathematical statistical developments, many of them made by Branch staff, promise to yield improved techniques in experimental design and analysis, particularly with respect to problems of carcinogenesis. The migrant studies have opened areas for development of the specific hypotheses on the etiology of gastrointestinal cancer. The Third National Cancer Survey will produce important data useful for epidemiological studies and for planning of cancer-control programs. The End Results studies have produced promising leads on prognostic factors in breast cancer and useful results may be forthcoming for other sites. Finally, we are now able to identify some members of the Branch staff who will be able to assume greater responsibilities for program direction and replace the present senior staff nearing retirement age.

Serial No. NCI-4250
1. Biometry Branch,
OASDD Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Activities during FY 1972

Previous Serial Number: Same

Principal Investigators: William Haenszel, Marvin Schneiderman, and Nathan Mantel

Other Investigators: Gary Glober, David Levin, James Koziol, James Murray, Charles Brown

Other NCI Collaborators: John Berg and Sidney Silverman

Man Years:

Total:	9.0
Professional:	6.5
Other:	2.5

Project Description

Objectives:

The principal objectives are to provide general planning and direction for the scientific program of the Biometry Branch, including recruitment and development of staff personnel and coordination of activities of statistical units assigned to other branches of NCI. The migrant population studies constitute a major part of the scientific program.

Methods Employed:

Standard methods of program planning, management, demography, biometry and epidemiology are applied to coordinate the Branch activities and conduct the research projects.

Major Findings:

The results of the current year's work have focused attention on the need for a continuous system for reporting cancer incidence in the United States with good regional balance. The conclusion of the reporting period of the Third National Cancer Survey has served to emphasize this need. An Ad Hoc Advisory Committee on Population-based Epidemiology Research Centers was convened and asked to

consider modification of the present program to include population-based registries for incidence reporting and subsequent epidemiologic research. The Committee recommended program changes in coordination with the National Cancer Plan and recommended that the new program consist of an optimum mixture of participating groups including registries in individual cancer specialty centers, population-based cancer registries, population-based cancer study centers, and comprehensive clinical cancer research centers. Program plans are in the process of being formulated to incorporate these recommendations.

The Migrant Studies Program currently consists of four components: Norwegian, Japanese, Colombian and Polish (supported by PL-480 funds in Poland). Data collection has progressed sufficiently in these studies to allow analysis of cancer trends in migrant groups. In all areas, efforts are directed toward obtaining comparative epidemiologic and supporting pathological data while exploiting the special capabilities of each collaborator and the special experiments of nature found in the different parts of the world. A review of epidemiologic findings in cancer of the colon and rectum and adenomatous polyps was published by Haenszel and Correa.

A paper summarizing the results of case-control studies of gastrointestinal cancer in Norway and Minnesota (for persons of Norwegian descent) is nearing completion. The findings with respect to associations with diet history and other characteristics are at best only suggestive but will have value when collated with the findings for other migrant groups, in particular Japanese migrants to the United States.

The cancer risks for Japanese migrants differ from those observed in Japan; the migrant risks are higher for lung, colon, rectum, prostate and lower for stomach while little change has been observed for breast. To date, the potential information exists tending to incriminate changes in dietary habits and in smoking habits for the changes in stomach cancer and lung cancer in the Japanese populations. Latent prostate cancer is almost as common in Japanese as in Americans though Japanese have little clinical cancer of this site. Japanese migrants to Hawaii have an intermediate position as to prevalence but show a complete shift in histology from "Japanese" to "American" type.

The findings from the Cali Mortality Survey were included in the initial UICC publication Cancer Incidence in Five Continents, and more comprehensive data were included in the second edition published in 1970. Cancer and premalignant lesions of the stomach have received special attention and the findings from the Cali cancer register and organ bank have been analyzed. This work has helped to clarify the possible role of intestinal metaplasia of the gastric mucosa as a premalignant lesion and contributed to the development of some hypotheses on pathogenesis. It is worth noting that these

findings display many points of consistency with observations made on Japanese migrants.

Dr. Schneiderman continued to be extensively involved in problems of safety and safety testing, including issues of pollution. He participated in an International Conference on the Statistical Aspects of Pollution Control, and upon the invitation of the International Statistical Institute, prepared a working paper for publication in Stockholm concurrent with the WHO sponsored International Congress on Pollution (June 1972). He is the project officer for a contract with the University of California at San Francisco for the development of new statistical techniques (including mathematical modelling of interactions of materials) for carcinogenesis testing. In relation to problems of epidemiology, he site-visted the IARC study centers concerned with EB virus and nasopharyngeal cancer at Hong Kong and Singapore. He participated in a UICC International Conference on Hodgkin's Disease.

In the past year, Dr. Schneiderman was named a member of the Board of Directors of the American Statistical Association and resigned his post as Invited Papers Editor of Biometrics. He remains on the faculty of the University of Pittsburgh and the Georgetown University Medical School, and continues as a member of the Council of the International Biometric Society, and as a member of the Scientific Advisory Board of the Veterans Administration Research Support Center, Washington, D.C.

Mr. Haenszel has devoted substantial amounts of time during the past year to consultation and planning for an expanded NCI program for support of epidemiological research. He is serving as a member of the Epidemiology Subcommittee of the Breast Cancer Task Force and as a member of the Committee for the Colon Cancer Segment established by the NCI Carcinogenesis Area.

Mr. Mantel has collaborated extensively with investigators in or formerly with the Epidemiology Branch (Glass, Oleinick, Miller, Li, Wertelecki, Priester, Fraumeni, Mulvihill), has assisted in the areas of both cancer chemotherapy and carcinogenesis (Goldin, Bates), and has advised other NCI investigators on numerous other problems. As an Associate Editor of Cancer Research, and as reviewer for other cancer journals, he has influenced the work of many other cancer investigators throughout the country. He has been consulted by staff of the Food and Drug Administration (Friedman, Gross) on problems of drug and food additive safety and has advised investigators at Oxford (Stewart, Kneale, Draper) on the reanalysis of their data-relating obstetric radiation to childhood cancer, an area of special concern to the Atomic Energy Commission (Hollister). He has been one of the advisers on the National Cancer Plan, consulting specifically with investigators at University of Waterloo, Ontario (Forbes) on cigarette-smoking effects and the development of less hazardous cigarettes. He has been active as a member of the Technical Advisory Committee

of the Federal Advisory Board to the Higher Education Panel operated by the American Council on Education. Miscellaneous statistical assistance has been provided to cancer investigators throughout the country who have had occasion to call on Mr. Mantel for advice. In addition, Mr. Mantel's work in technical statistical research has been mentioned at a high level including research on nonparametric procedures, contingency table procedures survival analysis, tests for association, iterative procedures. His assistance to other investigators doing such statistical research has been widely recognized.

Significance to the Program of the Institute:

This is the major biometric effort within the Institute. If the studies in the migrant populations elucidate the environmental factors involved in the incidence of certain forms of cancer and indicate those which are predominantly environmental and those which are predominantly genetic, then it may be possible to apply these findings to the United States population for purposes of cancer prevention and cancer control.

Proposed Course of Project:

Planning efforts will be directed toward development of a program consisting of an optimum mixture of various types of population-based cancer registries and cancer research centers. The recommendations of the Ad Hoc Advisory Committee on Population-based Epidemiology Research Centers will be utilized to the fullest extent possible. In the migrant studies we plan to delve much deeper into the pathogenesis of the major cancer types under study, especially cancer of the colon and rectum. Much greater effort must be directed to the synthesis of data in hand and for this effort to be successfully carried out, we must work more closely with the principal investigators in the field.

Honors and Awards

Correa, P.: Chairman, Committee on Geographic Pathology of the International Union Against Cancer.

Haenszel, W.: Member, American Cancer Society Advisory Committee on International Research. Member, Editorial Advisory Board, Environ Res. Adjunct Professor of Biostatistics, University of Pittsburgh Graduate School of Public Health. Member, American Association for Cancer Research Committee on Environmental Carcinogenesis. Member, Working Party on Neoplasms for U.S. Subcommittee on 9th Revision of International Classification of Diseases.

Levin, D.L.: Member, Clinical Trials Contract Review Committee, Chemotherapy, NCI.

Mantel, N.: Elected to Fellow, Institute of Mathematical Statistics; elected to membership, International Statistical Institute.

Schneiderman, M.A.: Member, Ad Hoc Committee, Advisory Committee to the Federal Radiation Council, Study of Uranium Miners, NAS-NRC. Member, Ad Hoc Committee on Evaluation of Low Levels of Environmental Chemical Carcinogens, DHEW. Advisory Panel on Carcinogenicity of Pesticides. Chairman, Animal Data and Human Studies, Subgroup IV, Tobacco Working Group, Lung Cancer Task Force, NCI. Member, Board of Directors, Washington Statistical Society. Member, Advisory Board, Research Support Center, Veterans Administration Hospital, Washington, D.C. Council Member, International Biometric Society. Chairman, Regional Advisory Board, Eastern North American Region, Biometric Society. Member, Board of Directors, American Statistical Association, Biometric Section. ASA Representative to the Regional Committee of the Biometric Society, ENAR. Member, Task Force on Aging Population. Adjunct Professor of Biostatistics, University of Pittsburgh Graduate School of Public Health. Clinical Associate Professor, Georgetown University School of Medicine. Reviewer for: Biometrics, Blood, Cancer Research, JAMA, Technometrics and Trans. Biomed. Engin., Science. Member, Ad Hoc Committee, Information Retrieval and Ranking of Estimated Risk for Potential Carcinogenic Hazards in Man, NCI.

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Serial No. NCI-4254
1. Biometry Branch, DCSDD
Etiology
2. End Results Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on Cancer in a Defined Population (Connecticut)

Previous Serial Number: Same

Principal Investigator: Roger Connelly

Other Investigators: William Haenszel, Sidney J. Cutler, William I. Lourie, Herman Heise, Lillian Axtell, Max Myers

Cooperating Units: Connecticut State Department of Health

Man Years:

Total:	3.0
Professional:	1.0
Other:	2.0

Project Description

Objectives:

To provide data on cancer incidence, methods of treatment, and end results; to investigate the effect on incidence of factors such as urban environment, nativity, economic status, occupation, diet and personal habits; and to study in detail the interrelationship between the condition of the patient at diagnosis, the therapeutic course, and the end results, in the general pract of medicine.

Methods Employed:

Hospitals accounting for all but 5 percent of the approved general hospital beds in the State participate in a case reporting and follow-up program administered by the Connecticut Health Department. Samples of cases reported to the registry are drawn for special study involving the examination of histologic specimens, and the collection of histories from patients and relatives.

Major Findings:

A major effort was put into preparation of data on individual patients for use in End Results Report No. 4. Time trends in intestinal and diffuse types of gastric cancer in Connecticut and the United States were studied and reported by Munoz and Connelly. Their findings support the hypothesis that

these two types of gastric cancer differ not only structurally and epidemiologically, but also etiologically. Through computer record linkage, Connelly and Christine found an increased risk of cancer for persons previously diagnosed with infectious mononucleosis when compared with the expected incidence based on the known cancer experience of the Connecticut population. A study of cancer of the lip in Connecticut, including incidence, treatment methods and survival, was completed and reported by Shedd, Von Essen, Connelly and Eisenberg.

Significance to the Program of the Institute:

The Connecticut Cancer Registry is the most extensive population-based program in the United States which provides reliable information on cancer incidence and mortality and information on the end-results of treatment. The completeness of case reporting and the efficiency of patient follow-up make possible the evaluation of the impact of case findings and treatment programs on the cancer problems as it affects a defined population. It provides a continuous resource for the study of cancer epidemiology and of the influence of social and biological factors on the end-results of treatment. It also provides a basis for evaluating the larger body of data obtained via the End Results Program.

Proposed Course of Project:

The proposed course is to develop an investigational team that will be able to fully exploit the research potential of the state registry. An active collaborative working arrangement must be established between the Connecticut Tumor Registry and the universities of the state. A variety of organizational formats are being considered, and several groups in the state are being consulted, e.g., the State Health Department, the State Medical Society, Yale University, the University of Connecticut, and the Connecticut Division of the American Cancer Society.

PUBLICATIONS

Munoz, N., and Connelly, R.: Time Trends of Intestional and Diffuse Types of Gastric Cancer in the United States. Int. J. Cancer 8: 158-164, 1971.

Serial No. NCI-4257
1. Biometry Branch, OASDD, Etiology
2. End Results Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Collaborative Studies of Cancer in Human Populations

Previous Serial Number: Same

Principal Investigators: Sidney J. Cutler, William Haenszel

Other Investigators: William I. Lourie, Jr., Lillian M. Axtell, Herman W. Heise, Roger R. Connelly, Max H. Myers, Ardyce Asire, Evelyn Shambaugh, Benjamin Hankey, Richard J. Kryscio, Stephen M. Baylor, Charles C. Brown.

Cooperating Units: California Tumor Registry; Connecticut Tumor Registry; Massachusetts Cancer Register; Charity Hospital of Louisiana; Indiana University Medical Center; State University of Iowa Hospital; University of California Medical Center; University of Chicago Clinics; University of Michigan Hospital; University of Virginia Hospital.

Man Years:

Total:	21.5
Professional:	16.0
Other:	5.5

Project Description

Objectives:

To assist other Branches of the National Cancer Institute, other health agencies, and treatment centers in the development and maintenance of programs for the collection and analysis of data on cancer in human populations and on the end results of treatment. This includes consultation and advice on methodological problems, development of improved statistical tools for the analysis of observations on human populations, and active collaboration in the collection and analysis of data in specific projects.

Methods Employed:

1. The End Results Section staff provides advice on the development and applications of uniform definitions and procedures for participating cancer registries, consultation on planning and implementing special studies, editing and analysis of data submitted to the National Cancer Institute in accordance

with a Uniform Code, and exploration of more efficient data processing and statistical techniques to abstract the maximum amount of useful information from the collected data.

2. The consultation draws on standard statistical and acturial techniques and accepted principles for records management, with necessary modifications required for any individual study situation.

Major Findings:

1. A firm answer to the question: "How many patients are being cured?" is unavailable because cancer can recur many years after treatment. The therapeutic success depends on diagnoses of cancer prior to spread beyond the site of origin. The most recent submission of data, as published in End Results in Cancer, Report No. 4, indicates that there are only a few sites for which there has been an increase in the percent of cancer diagnosed as localized since the 1950's:

Site	Percent Localized 1950-59	1960-64	1965-69
Female Breast	42	46	47
Prostate	52	60	63
Bladder	70	78	79
Melanoma of the skin	62	69	73
Brain	76	85	86

Survival data indicate continuing improvement for all these sites with the exception of breast cancer for which the 3-year relative survival rate has leveled off at slightly over 70 percent. Evidence of improvement in the management of cancer patients is indicated by improved survival observed for a larger list of sites despite the fact that for these other sites there is little evidence for "earlier" diagnosis:

3-year Relative Survival Rate (percent) for All Stages

Site	1950-59	1960-64	1965-69
Larynx	60	61	67
Prostate	59	64	66
Bladder	58	60	62
Melanoma of the Skin	63	69	74
Brain	28	33	37
Thyroid	81	85	86
Hodgkin's Disease	44	53	61
Multiple Myeloma	13	19	27
Chronic Lymphocytic Leukemia	49	50	53

It is encouraging that for a few sites around 90 percent of surgically treated patients without evidence of spread survive more than 5 years; e.g. 95 percent for uterine cervix and 97 percent for thyroid gland.

2. The 20-year survival rate, adjusted for normal mortality expectation, is a meaningful measure of the proportion of successfully treated patients. Twenty-year rates for patients treated recently can be projected by comparing survival curves for successive calendar periods. Available data permit projections for patients with cancers of the colon, rectum, bladder and female reproductive system. The estimated proportion of successfully treated patients is encouragingly high for those with resectable tumors and no evidence of spread beyond the site of origin: 85% for uterus, 75% for ovary, and 60% for colon, rectum, breast and bladder.

3. The correlation of structural features in the tumor and the regional lymph nodes with patient survival was confirmed in a study of 600 Norwegian patients with gastric cancer. Follicular hyperplasia in the regional nodes and lymphoid infiltration of the tumor are associated with longer survival, as are favorable nuclear grade and tumor grade determinations. Each of these factors is also associated with a more favorable extent of disease. In contrast with breast cancer, sinus histiocytosis of the regional lymph nodes is infrequently seen. The prognostic influence of the aforementioned factors could not be explained by taking histologic type into account. This study provides further evidence in support of a tumor-host interaction wherein a significant component of host response is mediated by lymphoreticuloendothelial system.

Significance to the Program of the Institute:

Many of the benefits of the consultation program are long range in nature and consist of setting up record systems and study situations which enlarge the potential study resources of NCI and related organizations. The research objectives of the Institute are promoted by continued work on the integration of various study techniques, e.g., retrospective and prospective studies, analyses of mortality data and morbidity surveys, epidemiologic investigation and cancer registries. An example of the interaction between epidemiologic and end-results investigations are the developing studies of prognostic factors and the role of these factors in cancer etiology.

Proposed Course of the Project:

1. A reorientation of the End Results Evaluation Program, placing greater emphasis on population-based registries, has been started. The California Tumor Registry is being reorganized in order to provide complete population coverage in five counties around San Francisco. Massachusetts is making good progress in developing a population-based registry in the Boston area. The possibility of utilizing the Third National Cancer Survey as a base for developing one or two additional population-based registries is being actively pursued. The goal is to develop several broad based research resources for a wide variety of studies.

2. Effective working liaison with Memorial Hospital (N.Y.C.) and with University of California School of Medicine in San Francisco is continuing. Possibilities for developing working relationships with other cancer special centers are being explored.

Publications

Zippin, C., Cutler, S.J., Reeves, W.J., and Lum, D.: Variation in Survival Among Patients with Acute Lymphocytic Leukemia. Blood 37: 59-72, 1971.

Black, M.M., Freeman, C., Mork, T., Harvei, S., and Cutler, S.J.: Prognostic Significance of Microscopic Structure of Gastric Carcinomas and Their Regional Lymph Nodes. Cancer 27: 703-711, 1971.

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Hankey, B.F. and Myers, M.H.: Evaluating Difference in Survival Between Two Groups of Patients. J.Chron.Dis. 24: 523-532, 1971.

Berard, C.W., Thomas, L.B., Axtell, L.M., Kruse, M., Newell, G. and Kagan, R.: The Relationship of Histopathological Subtype to Clinical Stage of Hodgkin's Disease at Diagnosis. Cancer Research 31: 1776-1785, 1971.

Mantel, N. and Hankey, B.F.: Programmed Analysis of a 2x2 Contingency Table. The American Statistician, 25: 40-44, 1971.

Cutler, S.J., Christine, B. and Barclay, T.H.C.: Increasing Incidence and decreasing Mortality Rates for Breast Cancer. Cancer, 28: 1376-1380, 1971.

Freeman, C., Berg, J.W. and Cutler, S.J.: Occurrence and Prognosis of Extranodal Lymphomas. Cancer 29: 252-260, 1972.

Mantel, N. and Myers, M.H.: Problems of Convergence of Maximum Likelihood Iterative Procedures in Multiparameter Situations. JASA 66: 484-491, 1971.

Serial No. NCI-4258
1. Biometry Branch,
OASDD, Etiology
2. Demography Section
3. Bethesda, Marylan

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Staff Studies of Cancer in Human Populations

Previous Serial Number: Same

Principal Investigator: Sidney J. Cutler

Other Investigators: Haitung King, Joseph Scotto

Cooperating Units: University of California Medical School
New England Deaconess Hospital
Lahey Clinic
Connecticut Tumor Registry

Man Years:

Total:	3.5
Professional:	1.5
Other:	2.0

Project Description

Objectives:

To describe cancer morbidity and mortality in human populations; to seek out and examine significant differences between persons who develop cancer and those who do not; to search for clues to causative or promoting factors of cancer by comparisons between high- and low-risk populations; to relate epidemiologic features of cancer to survival rates; to identify personal and environmental factors of potential significance in the prevention, control, early diagonsis, or successful treatment of cancer; and to present the results in an intelligible and usable form to medical students and practicing physicians as well as to other research workers.

Method Employed:

These studies require an extremely wide variety of methods of data collection and analysis, and in many cases new methods must be developed. Where appropriate, these are described in the following sections.

Major Findings:

1. Ad hoc studies being actively pursued now are limited because of the demands of the Third National Cancer Survey. Extensive analyses are being

completed of cancer morbidity and mortality among the Chinese in the People's Republic, Hongkong, Taiwan, Singapore, Hawaii, and the United States. While the research undertaking was planned several years ago, the forthcoming findings seem to be particularly useful in view of the current scientific interests in various aspects of medicine (including cancer) in Mainland China, and of prospective research opportunities in the People's Republic. The study of cancer morbidity is based on microscopically examined and/or registered cases during the past 50 years as specified below:

Microscopically Examined Cases

Peking - about 20,000 cases
Shanghai - about 30,000 cases
Taiwan - about 2,000 cases
Hongkong - about 4,600 cases
Singapore - about 8,000 cases

Registered Cases

Shanghai - about 20,000 cases
Hawaii - about 400 cases

For Mainland China, background information is given on highlights on cancer research and the anti-cancer campaign. Some of the achievements include the isolation of Actinomycin K from streptomyces malanochromogenes and its reported effectiveness on Hodgkin's disease patients, the remarkable progress during 1949-1968 in treatment of malignant trophoblastic tumors with the use of 6-Mercaptopurine, Methotrexate, 5-Fluorouracil, and Kengshengmycin, the completion of an analysis within eight days of nearly 30,000 tumor cases from among 150,000 surgical specimens spanning a 30-year period, the coverage in less than six weeks in a cervical cancer survey program (in a community of 100,000 population) of nearly 8,000 women over 30 years of age, and the claimed efficacy of some traditional drugs in producing in patients cyclical changes somewhat similar to those resulting from radiation therapy. The 5-year survivalship (percent) for cervical patients receiving radiation therapy was reported to be comparable to that of the United States.

Review and analysis of statistical data are pending. Some interesting associations include a possible link of high prevalence of esophageal cancer among Northern Chinese and consumption of ksoliang (a strong drink), the relevance of schistosomiasis in the development of colonic cancer, the occurrence of liver cancer during the intra-uterine life of the fetus, clonorchis sirensis as a liver carcinogen, a noticeably higher rate for female breast cancer among Mainlanders than among Taiwanese, a relatively high frequency of choriocarcinoma and of bone cancer, and an elevated prevalence of skin cancer in North China in relation to the customary use of Kang (oven-bed).

The study of cancer mortality deals mainly with U.S. Chinese, using data for 1959-1962 totaling 1,254 deaths. Supplementary data are incorporated into the analysis for Taiwan, 1959-1960 and 1963-1964, 19,202 deaths; Hongkong, 1961-1964, 10,810 deaths; Singapore, 1959-1962, 4,366 deaths; and British

Columbia, 1960-1962, 221 deaths.

Particular emphasis is placed on mortality differentials by nativity. Preliminary analysis indicated, for example, a lower risk for nasopharngeal cancer among U.S.-born than among China-born Chinese. Furthermore, the standardized mortality ratios for U. S. Chinese are intermediate between the high ratio in Hongkong and the low figure reported in Singapore and, particularly, Taiwan. The ranking of these areas seems to be consistent with what is known of the high risks for this cancer within China. Specifically, the majority of the Chinese in Hongkong originated in nearby Kwangtung Province, whose capitol provides the popular term for nasopharyngeal malignancy, i.e., "Canton Cancer". In contrast, most of the Chinese in Singapore are Fukienese who might have contributed to the low risk for this malignancy. Although the migrants from Canton predominate in the U.S. Chinese, the intermediate position might also have been the result of representation of non-Cantonese populations with lower risks. There seems to be a high degree of resemblance in risk patterns, as shown by morbidity and mortality findings, among the Chinese in various areas in regard to nasopharynx and other sites of cancer.

Significance to the Program of the Institute:

This program helps establish baselines for cancer mortality and morbidity in various populations from which changes can be measured, measures trends in cancer rates which may reflect the action of various etiologic agents, identifies high- and low-risk population groups which can be studied and compared, indicates areas for intensive efforts at case finding and follow-up, and provides for correlation of laboratory studies of cancer with observations on human populations. Ad hoc investigations are undertaken because of unusual or unexpected findings from the routine monitoring of incidence and mortality rates.

Proposed Course of the Project:

The immediate course of this project can not be predicted because many ad hoc studies are undertaken on short notice, as their need becomes apparent and as staff time is available. A number of studies are in various phases of completion, but the items listed below represent long-range goals and shortage of staff resources will not permit their completion and publication in the coming year.

1. Assembly of data for revision of Cancer Rates and Risks, a short book for practicing physicians, medical students, and others continues. This book has attained wide distribution since its publication in 1964, and we anticipate publication of another edition after the incidence data from the Third National Survey becomes available.

2. Data have been collected on non-cancer deaths among the Chinese in the same areas described under Major Findings. The forthcoming results of analysis should be useful in checking the validity of findings on cancer among the Chinese, such as the relationship between stomach malignancy and duodenal ulcer.

3. A study is being planned on consumption of herbs among the Chinese in several major centers of Chinese settlement including San Francisco, Singapore, Taiwan, and Hongkong. Field studies are being contemplated in Continental China in regard to selected cancer sites with high risk among the Chinese. Thoughts are also being given as to the possibility of collecting massive cancer data from various parts of New China.

4. Ten-year follow-up data has been obtained on approximately 3,500 polyp-free patients examined in the mid-1950's at the Lahey Clinic of Boston. The Lahey Clinic is conducting a survey of patients examined during the same time period but who were found to have rectal or colonic polyps. Results of the NCI portion of the study show a much lower than expected incidence of colon and rectal cancers in the polyp-free group. However, no conclusive or comparative statements can be made until the Lahey Clinic completes their portion of the study probably by mid-1972. Since there will be fifteen years of information on the polyp group, consideration will have to be given to collecting an additional 5-year follow-up data on the polyp free group. This could be conducted during the summer of 1972 if sufficient staff were available.

5. Approximately 1,000 cases of cancer of the prostate and urinary bladder and matched controls have been interviewed and the records coded, and analysis is well underway. Computer printouts have been obtained for two case study groups and three control groups. Analysis of data will begin under the following categories: Demographical information (i.e., race, place of birth, national origin, residence history, etc.); medical information (i.e., symptoms, treatment, related diseases, family history of cancer, cause of parents' death); social information (i.e., religious affiliation, marital history, lifetime sex activity, educational level, use of tobacco); dietal information (i.e., beverage usage, use of select foods and methods of preparation); occupational information (i.e., employment history, occupationally related exposures to carcinogens). Further analyses will develop from this to make use of other informational details which had been abstracted from the original interview schedules during the past year.

6. Investigation of mortality among defined cohorts of clergymen will be continued. Analysis to date demonstrates a reduced risk of death in all age groups and for most major causes of death, including many cancer sites. While the reasons for these reduced risks are not yet known, further accumulation of massive data at both national and international levels would provide useful clues for testing specific hypotheses. Such data collection could be quite profitable in view of the study results of disease-specific differences between Anglican clergymen in Britain and Episcopal ministers in the United States. Specifically, the former had a higher risk for arteriosclerotic and degenerative heart disease, cancers of the stomach and intestine, and hyperplasia of the prostate, but the reverse was true for leukemia, prostatic cancer, diabetes, and suicide. Perhaps comparative studies of other clerical groups at the cross-cultural level could enlighten us further regarding the above variations. Accordingly, the clerical study is moving toward comparisons of mortality levels between the Scottish Presbyterian clergymen and U. S. United Presbyterian ministers.

Honors and Awards

King, Haitung: Elected a Fellow, the Royal Society of Health. Advisor-Consultant, D. C. Interagency Council on Smoking. Invited to contribute a chapter on "Selected Epidemiological Aspects of Cancer in New China" in a monograph entitled Reference Handbook in Chinese Medicine to be published by the Fogarty International Center.

Publications

King, H.: Clerical Mortality Patterns of the Anglican Communion. Soc. Biol. 18:164-177, 1971.

Scotto, Joseph: Coffee-Drinking and Bladder Cancer, letter to Lancet, Nov. 27, 1971 (with Joseph F. Fraumeni, Jr. and Lucia J. Dunham).

Serial No. NCI 4260
1. Biometry Branch, OASDD, Etiology
2. Demography Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Statistical Consultation for the Veterans Administration Urological Research Group

Previous Serial Number: Same

Principal Investigator: David P. Byar

Other Investigator: Kenneth S. Hurst

Cooperating Units: V. A. Urological Research Group (21 hospitals)

Man Years:

Total:	3.5
Professional:	2
Other:	1.5

Project Description

Objectives:

To provide consultative services in statistical and epidemiological methodology necessary for the various programs sponsored by the Veterans Administration Urological Research Group. This includes assistance in the design, interpretation and evaluation of the clinical trials conducted by the Veterans Administration Urological Research Group, and provision for studies operating concurrently with the clinical trials such as epidemiological studies.

Methods Employed:

Standard methods of biometry, statistics, probability, and epidemiology with necessary modification as required by the particular problem. New techniques are developed by the personnel working with the Section to handle specific problems.

Major Findings:

This Section assists the investigators in developing and evaluating methods of treatment for cancers of the male genitourinary tract. Such assistance is generally acknowledged in the publication of findings of the medical investigators. New statistical techniques are published independently.

The Section is more deeply involved in six separate clinical trials:

(a) Main Prostate I, which compares the effect of two treatments (prostatectomy alone, and prostatectomy with estrogen) for Stages I-II, and of four treatments (estrogen, orchiectomy, estrogen plus orchiectomy, and placebo control) for Stages III-IV. The intake of new patients into this study was terminated on March 31, 1967. A total of 2,315 patients had been entered by that date.

(b) Main Prostate II, which also compares the effect of two treatments (prostatectomy alone and placebo control) for Stages I-II and of four treatments (three levels of estrogen and placebo control) for Stages III-IV. This study is designed as a sequel to Main Prostate I. Intake of new patients was terminated in June 1969 after 561 patients had been entered in the study.

(c) Main Prostate III, which compares placebo versus prostatectomy for operable Stage I-II patients. Inoperable Stage I-II patients are randomly assigned to placebo or 1.0 mg. Diethylstilbestrol. All eligible Stage III-IV patients are randomized to one of four treatments: 1.0 mg. Diethylstilbestrol, 30 mg. Provera, 2.5 mg. Premarin, or 30 mg. Provera plus 2.5 mg. Premarin. As of September 1, 1971, 557 patients had been entered in this study.

(d) Focal Carcinoma, which compares several treatments for very early prostatic lesions of the type commonly found in TUR or autopsy specimens from old men. There are now 175 patients in this study.

(e) Urinary Bladder I, which compares surgery alone, radiation alone, and combined therapy for bladder cancer. There have been 72 patients admitted.

(f) Urinary Bladder II, which compares transurethral surgery followed by oral placebo, oral pyridoxine, or topical installation of thio-TEPA in treating Stage I tumors of the bladder. Intake of new patients into this study was begun in November 1971.

In addition to these six clinical trials, the VA group has several other studies for which the National Cancer Institute provides consultation as requested.

The data from two trials -- Main Prostate III, and Urinary Bladder II -- are not yet sufficient for detailed study.

The results of Main Prostate I have already caused widespread changes in the general treatment of prostatic cancer, because of the finding that the standard estrogen treatment for prostatic cancer carries a very great risk of death from cardiovascular diseases. In addition, the clinical benefits with respect to the cancer are smaller than was generally believed. Overall mortality rates in Stages I, II, and III, and in "early" Stage IV are substantially higher in estrogen-treated patients than in those not receiving estrogens. This hazard was not recognized before the results of this study were available.

During the past year, efforts in this project have been concentrated on three objectives: elucidation of the mechanisms by which estrogen exerts its harmful effects, classification of patients with respect to the probable benefits or losses associated with each treatment tested, and studies (Prostate II) on the effects of various doses of diethylstilbestrol.

Preliminary results from Main Prostate II indicate (1) that there is no evidence thus far to show that 5.0 mg. diethylstilbestrol is superior to 1.0 mg. in controlling the cancer in either Stage III or IV, (2) that in Stage III there are substantially fewer heart and cardiovascular deaths on 1.0 mg. diethylstilbestrol compared to the 5.0 mg. dose, (3) that in Stage IV both the 1.0 mg. and 5.0 mg. doses are more effective than the 0.2 mg. dose in controlling the cancer, and (4) that in Stage III the superiority of placebo over the 5.0 mg. dose (and to a lesser extent over the other two doses of diethylstilbestrol) is accounted for by the excess deaths from non-cancer causes in the estrogen treated patients. Taking all these rather complex relationships into account we see no justification for the continued use of 5.0 mg. diethylstilbestrol in either Stage III or IV.

At the time that Main Prostate I was organized there was no reason to suspect an adverse effect of estrogen on the cardiovascular system, so much important information was not collected and submitted in a uniform way. We are now attempting to gather some items retrospectively (pre-treatment EKG, history of heart disease, admission blood pressure, etc.) but the analysis will be difficult. Main Prostate II includes very careful study of the cardiovascular system before treatment and at each follow-up, and may eventually provide answers to this problem. We are currently studying this problem by means of a multivariate Bayesian analysis using ten pre-treatment variables including a number of laboratory values (e.g. cholesterol, fibrinogen, plasminogen) and clinical measurements (e.g. weight and blood pressure). Completion of this work will require extension of existing statistical methodology and testing of the results by half-replication techniques.

It must be noted that cardiologists have not accepted the estrogen findings as readily as urologists. Results of the VA study have been of concern to the National Heart and Lung Institute because of its involvement in the Coronary Drug Project in which several drugs (including estrogen) are actually being used in the hope of reducing mortality among men with a recent myocardial infarct. NHLI assigned a staff member to make a detailed review of our original records and other data on Stages I-II, where the adverse effect of estrogen is most marked. His review completely supported all of our published conclusions. In November 1970 the first report of the Coronary Drug Project appeared (JAMA 214: 1303, 1970). In this report, data were presented which indicated that 5.0 mg. conjugated estrogen daily led to a significant increase in non-fatal events of myocardial infarction, pulmonary emboli, and thrombophlebitis compared to the placebo group. This treatment has since been dropped from their study.

During the last year we have analyzed the data from the Focal Carcinoma study and have found that almost none of the patients have died of cancer of the prostate. We are currently engaged in adjustment procedures to see if the survival of these older patients who did not have radical mastectomy is as good as that of the comparable group of younger patients from Study I who did, when age is taken into account. Our paper on the Focal Study has been accepted to be read to the Annual Meeting of the American Urological Association in May 1972.

Analysis of the Urinary Bladder I Study revealed that no difference was apparent in the three treatment groups being compared. However, there were many flaws in the study, and the number of patients entered was small. We are currently preparing a paper on the results, both to report our negative findings, and to point out some of the difficulties of conducting a successful clinical trial in this rapidly progressing disease.

Significance to the Program of the Institute:

This Section serves the members of the Veterans Administration Urological Research Group by assisting in preparing and revising protocols, forms, and records; giving statistical guidance in the design and execution of clinical trials; processing and analyzing data that is received daily on patients in the various studies; and assisting the members of the Group with reports on the progress of the various studies.

In return for these services, the Demography Section has access to the largest complete set of data available anywhere on the course of prostatic cancer. Over 2,300 patients have been entered in Main Prostate I with follow-up as long as ten years. These data are proving extremely useful and interesting not only in the comparison of treatment effects, but also in defining the general behavior of prostatic cancer in relation to a host of personal, clinical, and laboratory characteristics.

Proposed Course of the Project:

The cooperative studies undertaken jointly by the Veterans Administration and this Section are planned to be a self-generating sequential series to continue as long as fifteen years. Consultative services of this Section have expanded considerably over the past year as the data from Main Prostate I and II have ripened and as patients have accumulated in Main Prostate III.

In the coming year, we will continue to give most of our attention to the analysis of Main Prostate I and II, and especially of the patients in Stages III-IV, since the other studies do not yet include large numbers of patients with long-term follow-up. We are also using the data we have collected to study several methodological aspects of the analysis of data from clinical trials. Specifically we are studying the problem of how best to analyze the data when not all patients remain on the assigned treatment in randomized trials, how to compare the secondary treatments when second phase treatment was not part of the original design, and we are making power comparisons of various tests used in comparing survival curves.

Studies of specific cardiovascular effects of estrogen on patients in Main Prostate I have been undertaken, but the data are scanty and difficult to interpret. Main Prostate II was designed to include detailed heart studies, but it will take several more years to collect sufficient data for a full analysis.

Because the amount of useful data collected in these studies is more than our present staff can adequately analyze, contracts for assistance in the analysis were offered to the Yale University Department of Epidemiology and Public Health. During the past year and a half extensive work has been done extending work originally begun by us on the use of a multivariate exponential survival model. Specifically, we have studied a step-wise procedure for eliminating variables from the analysis in order of their importance in predicting survival based on the likelihood function. A goodness of fit test has been devised for checking the agreement of the model with the observed data, and the model has been tested by means of half-sample pseudoreplication. These results are currently being prepared for publication. We have also investigated the effects of including first order interactions in the model, and have found that with these data they do not add appreciably to the information. We have compared estimates of the variance of the parameters of the model based on the asymptotic variances with those obtained in half-sample pseudoreplication and found that when estimating three such variances on samples of only 100 observations, the two estimates were in good agreement. It is expected that this work will continue for a third contract year starting in July 1972, and that it will be completed in that year.

Since last August we have been able to intensify our efforts to analyze the large amounts of data at our disposal because of programming assistance from the Wolf Research and Development Corporation provided us as part of a general contract for the Demography Area. We are currently using two full-time programmers and this relationship has been surprisingly convenient and productive.

Publications

Byar, D. P., and The Veterans Administration Cooperative Urological Research Group: The treatment of prostatic cancer: Studies by the Veterans Administration Cooperative Urological Research Group. Bull. New York Acad. of Med. (In press).

Byar, D. P.: Zinc in male sex accessory organs: distribution and hormonal response. In Structure and Function of the Male Sex Accessory Organs (Brandes D, ed.). Academic Press Inc. (In press).

Byar, D. P., Mostofi, F. K., and The Veterans Administration Cooperative Urological Research Group: Carcinoma of the prostate: prognostic evaluation of certain pathological features in 208 radical prostatectomies. Accepted by Cancer.

Huse, R., Byar, D. P., Bailar, J. C., III, and The Veterans Administration Cooperative Urological Research Group: A multivariate exponential model for predicting survival of prostatic cancer patients with time-censored data. Submitted for publication to JNCI.

Serial No. NCI-4265
1. Biometry Branch, OASDD, Etiology
2. Mathematical Statistics and Applied Mathematics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Consulting in Statistics and Applied Mathematics

Previous Serial Number: Same

Principal Investigators: John J. Gart, Hugh M. Pettigrew, Robert J. Connor, Donald G. Thomas

Other Investigators: Jun-mo Nam, Alroy M. Smith

Cooperating Units: None

Man Years:

Total:	3.5
Professional:	3.0
Other:	0.5

Project Description

To provide consulting assistance and collaboration to NCI scientists on the applications of mathematical statistics and applied mathematics to the interpretation and analysis of scientific experiments.

Objectives:

The principal objectives are (1) to collaborate with NCI scientists on mathematical problems related to cancer research; (2) to provide consulting assistance in statistics and applied mathematics to NCI investigators; and (3) to accelerate the use of quantitative methodology in various aspects of the NCI intramural program and extramural program.

Methods Employed

The methodology of applied mathematics, mathematical statistics and probability are applied to biomedical problems. Often variations of existing techniques are developed to suit the special requirements of a

particular problem. The members of this section consult with a great many investigators in all areas of intramural research in the NCI.

Major Findings:

During this year the staff consulted with many different branches and laboratories of the NCI sometimes with several investigators in an individual branch or laboratory. In addition the staff has consulted with other researchers at other Institutes of N.I.H.

Dr. Gart has been directing the statistical analysis of a cigarette condensate painting experiment done on contract at Hazleton Laboratories. Mr. Thomas and Mrs. Smith of this section and Mrs. Staneck of the DCRT have been constructing the rather extensive and complex computer programs required to perform the statistical analysis. The study involves 7,000 mice observed for eighteen months. Life table analyses, with graphical output, censored rank order tests and Weibull survival curves are among the methods used in this analysis. Dr. Gart has also been an active participant in discussions relating to the design and planning of a second such large experiment.

In relation to the tobacco experiment Mr. Thomas has been developing computer programs for fitting Weibull survival curves to right-censored data. This model has proved very useful in fitting data for a variety of carcinogenic agents in animal experiments.

Dr. Pettigrew, with Dr. David Kaufman, of the Lung Cancer Unit, Carcinogenesis Area, designed a large experiment to study the effectiveness of two treatments in reducing tumor incidence in hamsters treated intertracheally with a carcinogen known to cause respiratory tract tumors in virtually all animals at the dosage used. The design had to take into account such things as the fact that the animals were of three different ages, the carcinogen had to be mixed fresh several times during each day the animals were treated, and each batch of carcinogen might decline in potency as it was being used. This experiment is still going on, and the data will be analyzed as it becomes available. Also with Dr. Kaufman, Dr. Pettigrew is currently analyzing data from several completed experiments to test various carcinogens administered I.T. for their ability to induce respiratory tract tumors. Dr. Pettigrew and Dr. Kaufman have been working for several months on a paper to describe, in layman's terms, methods for analyzing data (from carcinogenesis experiments) that adjust for competing risks, e.g. the Kaplan-Meier method and the Breslow test.

At the request of Dr. Ian Mitchell of the Surgeon General's Office, Dr. Connor collaborated with Drs. Umberto Saffiotti, John Cooper, and Norbert Page, Carcinogenesis, NCI in a review and analysis of experiments carried out by Procter and Gamble on the effect of NTA on mice. On this project Dr. Connor was joined by Drs. David Hoel and Michael Hogan, Biometry Branch, NIEHS, in the statistical analysis and the writing of a statistical report which was submitted to the Surgeon General. In addition, he participated in Conferences held by the Surgeon General to discuss NTA and its biologic and environmental effects.

Dr. Gart and Mr. Nam collaborated with Dr. Rogentine of the Immunology Branch on a large study of the frequencies of HL-A blood frequencies among disease and normal groups. The major study showed the frequency of HL-A2 was significantly higher among 50 children with acute lymphocytic leukemia than among 200 normal controls. The study is a subject of a paper accepted for publication of which Dr. Gart and Mr. Nam are co-authors. Mr. Nam and Dr. Gart also analyzed preliminary data on studies relating HL-A blood groups with other diseases with Dr. Rogentine as well as with Dr. Whisnant of NICHD.

In connection with these studies Mr. Nam has developed a computer program for estimation of gene frequencies and discrepancy chi-square test in a complex analysis of HL-A blood groups. Mr. Nam and Dr. Gart have been also working the distribution of HL-A frequencies among families of leukemia patients by using Elandt-Johnson methods of estimation.

Dr. Pettigrew consulted with several members of the Surgery Branch about a number of projects. In collaboration with Dr. Samuel Wells, he is analyzing data from a series of patients operated on for adenomas of the parathyroid gland, attempting to relate tumor volume to pre-operative serum levels of calcium and PTH. This relationship is of clinical importance, since it would be of great benefit to the surgeon to be able to predict tumor size, these tumors being rather difficult to locate. With Dr. William Catalona, Surgery Branch, Dr. Pettigrew is performing an analysis of a large amount of data gathered from a series of cancer patients and normal controls to determine whether or not they differ with respect to certain measurements on their serum. Among the various variables that may also affect this are age, sedimentation rate, and hematocrit. With Dr. Mark Soloway, Surgery Branch, Dr. Pettigrew is analyzing an experiment to determine the effect of various drugs on the growth of a transplantable murine tumor as evidenced by proportions of animals developing tumor and growth rates of tumor. A second project with Dr. Soloway relates to an experiment involving a carcinogen-induced

murine bladder tumor in order to determine the toxicity of the carcinogen as evidenced by survival time. With Dr. Roger Millar, Surgery Branch, Dr. Pettigrew recently discussed design of a guinea pig experiment to study the effect of BCG on tumor growth, and to compare the potency of BCG from two different sources. The resulting data will be analyzed when the experiment is completed. With Hilda Wexler of the Surgery Branch, Dr. Pettigrew has analyzed data from numerous experiments dealing with metastatic behavior of animal tumor systems and its relationship to host immunity. Presently he is now helping Miss Wexler compile a summary of the main results obtained by herself and Dr. Ketchum over the last ten years.

Mr. Nam consulted with Dr. E.O. Reiter, Reproduction Research Branch, NICHD on studies of the survivorship of 240 newborn sick babies. They studied the effect of blood transfusion on the chance of survival when adjusted for birth weight, initial blood pressure, HCT, and other factors.

Dr. Connor consulted with Dr. Eugene B. Rosenberg, formerly Laboratory of Cell Biology, NCI who requested advice on statistical treatment of outliers in ongoing study of human viral leukemia utilizing in vitro tests. After discussion of entire problem, Dr. Connor recommended a complete program for the statistical analysis of their test results. Dr. Connor aided Dr. Joel M. Engelstein, Laboratory of Cell Biology, NCI, and reviewed the statistical analysis and presentation of his data on corneal allografts. Results of this study are reported in a paper by Dr. Engelstein and his associates entitled "Protection of Corneal Allografts from Immune Rejection."

Dr. Connor is consulting with Dr. Curtis Harris, Carcinogenesis, NCI on statistical aspects for contract work being done in the Dept. of Pathology at the University of Utah. This project is concerned with immunosuppressive drugs and their relation to carcinogenetic agents in mice and rats. Dr. Connor is advising on statistical analysis of data for current experiments and advising a design of future work. Possibly Dr. Connor will do analysis for this study as well as make a site visit.

Dr. Connor, working with Drs. William Terry and Steven Rosenberg, Immunology Branch, NCI and Dr. David Lavrin, Bionetics, did the statistical analysis for study of BCG effects on the induction of tumors for mice given MCA . He is currently carrying out life table analysis and advising on future experimental work.

Dr. Connor, working with Dr. Chou-Chik Ting, Cellular and Tumor Immunology Section, Laboratory of Cell Biology, NCI, advised and did statistical analysis on research reported in a paper entitled "Tumor Specific Antigens in Polyomavirus Induced Tumors" by Dr. Ting and his associates.

Dr. Connor consulted with Dr. Mary Colman, formerly of Children's Hospital on the analysis and presentation of data for a study of Down's syndrome.

Dr. Connor reviewed the statistical analysis of a large scale study of the carcinogenicity of several cigarette condensates performed by A.D. Little.

Dr. Connor has advised several scientists for the Biology Branch, NCI (e.g. Drs. Berton Zbar, Bruce Littman and E.B. Chung) on problems in the statistical design and analysis of their experiments.

Mr. Nam consulted with Dr. Woo of Chemotherapy, on a method of detecting the baby's sex in a pregnant woman.

Dr. Gart has been collaborating with Dr. J.H. Robbins of the Dermatology Branch on a study of the millipore assay technique for measuring tritiated thymidine incorporation into DNA on leukocyte cultures. Dr. Gart is the co-author of a paper on this work soon to appear. Other work with Dr. Robbins on this work relates to the"clumping" or lack of random scatter of mononuclears on coverslips.

Dr. Gart has collaborated with Dr. Whang Peng of the Human Tumor Cell Biology Branch on studies of cell cycle characteristics in leukemic patients. He is co-author of a paper in Blood on this work.

Dr. Gart has consulted with Dr. Mearl Stanton on problems of mesothelioma induction by various materials in animal experiments.

Dr. Pettigrew consulted with Dr. Stuart Siegel of the Medicine Branch on the final analysis of data from the "life-island" trial: a clinical trial designed to evaluate the usefulness of protected environments in the treatment of leukemia patients.

Dr. Pettigrew advised Dr. Gary Pearson of the Viral Biology Branch on the proper method of analyzing data from a lymphocyte toxicity test. He also analyzed data from an experiment comparing normal and immune lymphocytes with respect to cytotoxicity.

Dr. Pettigrew for Dr. Willie Turner and Mrs. Leslie Bass of the Viral Leukemia and Lymphoma Branch, analyzed data from a study of a semi-micro XC cell assay technique for murine leukemia virus. With Dr. Michael A. Chirigos and Dr. John W. Pearson, of the same branch, he has performed a preliminary analysis of data obtained from an experiment currently in progress to determine the effects of prophylactic adjuvant treatment with BCG and C. gran. on survival of AKR mice. With Dr. John A. Pearson, of the Viral Leukemia and Lymphoma Branch, Dr. Pettigrew analyzed data from a study of combination chemo-immuno-therapy against LSTRA.

With Dr. Pietro Gullino of the Laboratory of Biochemistry, Dr. Pettigrew has analyzed various sets of data obtained from animals with growing and regressing tumors, in order to study the phenomenon of tumor regression.

With Dr. David Levin, Biometry Branch and Dr. Stephen Carter, Cancer Therapy Evaluation Branch, Dr. Pettigrew participated in the evaluation and redesign of a proposed grant for a clinical trial to evaluate the degree of hepatoxicity associated with the treatment of psoriasis with methotrexate.

Mr. Nam consulted with Dr. W.R. Levis, Dermatology Branch on the analysis and presentation of data on the methods for obtaining a human peripheral blood leukocyte.

Alroy M. Smith plays an important supportive role in the data analysis of this section. In particular, in the past year she has converted several programs useful in analyzing survival curves in clinical trials and animal experiments to the NIH system. These have and will prove useful in analyzing many of the studies reported elsewhere in this report. Mrs. Smith has also recently completed a more intensive statistical analysis of the Bionetics Study of Pesticides.

Mr. Thomas served as the section' s "expert" on high speed computing often consulting with other members of the section and branch on many projects which depend on computers. He is developing a "library" of computer routines which will prove useful in the consulting activities of other members of the section. A great deal of his time this year has been spent in facilitating the transfer of this section' s computing to NIH' s 360/370 system via our new IBM 2741 remote terminal.

Dr. Connor has been advising Dr. Manfred M. Mayer, Microbiology Department, School of Medicine, Johns Hopkins University, on a proposed procedure involving "proportional windows" for counting cells using a Coulter Counter.

Significance of the Problem to the Institute:

Members of this section are assuming an essential role in much research within the NCI. Their activities include not only statistical analysis but also in planning valid experiments.

Proposed Course of the Projects:

Several of the projects mentioned in the Major Findings section will continue. In particular the consultation with the chemical carcinogenesis and the association with the tobacco painting research is expected to continue.

Honors and Awards

Dr. Gart continued to serve as a member of the Biometric and Epidemiological Methodology Advisory Committee of the FDA.

Dr. Pettigrew was an invited participant in the Workshop on the Statistical Interpretation of Mammalian Survival Experiments held in Gatlinburg, Tenn. in October 1971.

Dr. Connor was appointed as a reviewer of contract proposals submitted to the Adult Development and Aging Branch of the NICHD.

Dr. Pettigrew was an invited participant in the International Workshop on Psoriasis-Liver-Methotrexate Interaction held at the University of Miami in November 1971.

Dr. Connor reviewed several papers for the Human Genetics Branch of NIDR.

Dr. Connor served on a site visit team on the statistical design and analysis of a grant proposal at Oak Ridge for the NICHD.

Dr. Gart served as a referee for Science. Dr. Connor, Dr. Pettigrew, and Dr. Gart served as referees for J. N. C. I.

Publications

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Kagan, A.R., Hamilton, M.A. and Bryant, T.L.: Radiosensitivity of in vivo mouse bone marrow cells. Radiologia Clinica et Biologica, 142-149, 1971.

Kagan, A.R., Hafermann, M., Hamilton, M., Pierce, R., Morton, D. and Johnson, R.: Etiology, diagnosis, and management of pericardial effusion after irradiation. Radiologia Clinica et Biologica, in press, 1972.

Robbins, J.H., Gart, J.J., Levis, W.R. and Burk, P.G.: The millipore filter assay technique for measuring tritiated thymidine incorporation into DNA in leukocyte cultures. Clin. Exp. Immun., in press, 1972.

Rogentine, G.N., Jr., Yankee, R.A., Gart, J.J., Nam, J. and Trapani, R.J.: HL-A antigens and disease. I. Acute lymphocytic leukemia. J. Clin. Inv., in press, 1972.

Slivka, J., Cargille, C.M. and Ross, G.T.: An analysis for superimposed biological rhythms: application to FSH and LH in women. Am. J. Physiology 30, 905-908, 1971.

Whang-Peng, J., Perry, S., Knutsen, T.A., and Gart, J.J.: Cell cycle characteristics, maturation, and phagocytosis in vitro of blast cells from patients with chronic myelocytic leukemia. Blood 38: 153-161, 1971.

Serial No. NCI-4267
1. Biometry Branch, OASDD, Etiology
2. Mathematical Statistics and Applied Mathematics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Research in Mathematical Statistics and Applied Mathematics

Previous Serial Number: Same

Principal Investigators: John J. Gart, Robert J. Connor, Hugh M. Pettigrew, Donald G. Thomas

Other Investigators: Jun-mo Nam, Alroy M. Smith

Cooperating Units: None

Man Years:

Total:	3.5
Professional:	3.0
Other:	0.5

Project Description

Objectives:

To conduct basic research in mathematical statistics, probability and applied mathematics; to develop new statistical methodology which is especially appropriate to bio-medical sciences.

Methods Employed:

The methods employed are the modern theories of mathematical statistics, probability and applied mathematics. High speed electronic computers are often used to compute appropriate mathematical tables and test approximations by simulation techniques.

Major Findings:

The Research of the members of this section covers a wide spectrum of topics in mathematical statistics, probability and applied mathematics. These are summarized below.

1. Interaction in 2 x s x t Contingency Tables.

John J. Gart devised a simple method for testing higher order interaction in 2 x s x t contingency tables. The method, considerably simpler numerically than previously proposed methods, will be published shortly in Biometrika. It will prove useful in deciding whether related experiments comparing proportions may be validly combined.

2. Grouping and Testing for Trend in Categorical Data.

Robert J. Connor completed a paper on grouping for testing trends in categorical data. This paper will soon be published in The Journal of the American Statistical Association. He is continuing his investigation of the small sample properties of the exact test for ungrouped data versus the usual approximate statistical test.

3. Models for Investigating Procedures in Carcinogenicity.

Hugh M. Pettigrew and Robert J. Connor are investigating deterministic and stochastic models for carcinogenesis experiments, with particular regard to their implications as to proper methods of analysis, including adjustments for lost (either escaped or cannibalized) animals. These models should be very useful in the interpretation of carcinogenesis experiments in which the lesion of interest is not observed until the animal dies, and causes of death are not assigned. Dr. David Kaufman of the Carcinogenesis area will provide advice as to the biological validity of the assumptions made.

4. Validity of Nominal Confidence Limits in Bioassay with Small Samples.

Jun-mo Nam has been investigating the adequacy of nominal confidence limits calculated from the minimum logit χ^2 method and its resulting asymptotic properties, in order to delimit explicitly when the procedure can be validly used for small samples.

5. Exact Test and Confidence Limits for Combining 2 x 2 Tables.

Donald G. Thomas is currently developing a computer program to compute tests and exact confidence limits for the common odds ratio in combined 2 x 2 tables. This work is in the final stages of development and should be submitted for publication shortly.

6. A Simple Approximate Method for Relative Risk Analysis.

Donald G. Thomas and John J. Gart are completing research on a modification of Gart' s previously proposed approximate method for finding confidence limits for the odds ratio or relative risk.

7. Numerical Results on Relative Risk Analyses.

John J. Gart and Donald G. Thomas have completed work on a paper investigating various approximate methods for finding confidence limits on the relative risk or odds ratio. Their paper will soon appear in The Journal of the Royal Statistical Society.

8. Test of Several Treatments vs. Control for Proportions.

Robert J. Connor and Hugh M. Pettigrew are investigating methods of analysis of data where several observed proportions are to be compared to a control proportion. This problem would arise, for example, in situations in which the biological observation made was the proportions of animals in an experimental group that developed tumors.

9. "Jackknifing" Genetic Estimators.

Donald G. Thomas is collaborating with Nathan Mantel on the improvement in bias of certain estimators useful in human genetics by the so-called jackknife method.

10. Covariance Matrices Tests.

Robert J. Connor has completed research with Richard L. Greenstreet of the Johns Hopkins University on the power of some common tests for the equality of covariance matrices for the multivariate normal case. A paper has been submitted for publication.

11. Research in Epidemic Models.

Hugh M. Pettigrew is continuing his research into deterministic and stochastic models to describe the spread of epidemics. Extensions of the existing theory include simple deterministic and stochastic models with two or more types of infectives, and a generalization of the Pettigrew-Weiss large-population model to allow for k-types of infectives.

12. Reed-Frost Epidemic Models with Several Kinds of Susceptibles.

John J. Gart completed his research on the extension of Reed-Frost epidemic models to the case of several kinds of susceptibles. A paper on this work has been submitted for publication.

13. Sample Sizes in Comparison of Proportions.

John J. Gart has collaborated with Dr. Mitchell Gail of the Viral Biology Branch on the preparation of a more precise table for deciding on sample sizes in experiments comparing proportions.

14. Optimum sample sizes for the comparison of control and treatment means.

Jun-mo Nam is revising this paper on the optimum sample size which may help reduce the number of inconclusive and wasteful experiments.

Significance to the Program of the Institute

The interplay between mathematical theory, data analysis and experimental research is an important element in biomedical research. Many of the "Major Findings" reported above are new statistical techniques which have or may be directly applied to data collected by the medical researchers at NCI or other workers on problems in cancer research. Others are mathematical models which may elucidate the biological phenomena involved in cancer research and which may also aid in the planning of subsequent experiments or epidemiologic studies. The opportunity for initiating fundamental research on mathematics and mathematical statistics is essential for enabling members of the section to achieve professional recognition among their peers in their own scientific disciplines. More importantly, the possibility of doing such unconstrained research is a prerequisite for the consulting work of the section to be carried out at the highest professional level.

Proposed Course of the Project:

Many of the projects described in the Major Findings will be continuing. Such subjects as exact statistical methods in bioassay and epidemic theory will be investigated by various members of the section. Other problems, not referred to above, shall also be attacked in the upcoming year: they will include the development of new statistical methods and mathematical models in various biomedical problems, particularly in the area of models for the analysis of survival curves in carcinogenesis experiments.

Honors and Awards

John J. Gart was elected a member of the Regional Committee of the Biometric Society.

Robert J. Connor was elected an Honorary Fellow of the A.A.A.S.

John J. Gart was appointed program chairman of the Biometric Society for its 1972 National Meeting in Montreal, Canada.

John J. Gart was appointed Associate Editor of The American Statistician.

John J. Gart was a member of the Conference Record Committee of the International Statistical Institute' s Meeting in Washington in August 1971. Hugh M. Pettigrew and Robert J. Connor served as recorders of the scientific discussion at this meeting.

John J . Gart served as consultant to several granting institutions in judging research grants in mathematical models and statistics. The granting institutions are the Program for Applied Mathematics and Statistics of the N.S.F., The Computer and Biomathematical Sciences Study Section, Division of Research Grants, N.I.H., and the Pure and Applied Mathematics Grants Selection Committee, National Research Council of Canada.

John J. Gart gave an invited paper entitled "The statistical analysis of chain-binomial epidemic models with several kinds of susceptibles" at the annual meeting of the American Statistical Society at Fort Collins, Colorado in August 1971.

John J. Gart gave an invited talk on the "Review of the theory and application of methods for comparison of proportions" to the 17th Conference of the Design of Experiments in Army Research at Walter Reed Hospital in October 1971.

John J. Gart gave an invited lecture at Rice University in Houston to the Southeast Texas Chapter of the American Statistical Association in November 1971.

John J. Gart served as referee for Biometrika, Biometrics and The Journal of the American Statistical Association. Dr. Pettigrew served as a referee for The American Statistician.

Publications

Connor, R.J., Grouping for testing trends in categorical data. Jour. Amer. Stat. Assoc., 67: in press, 1972.

Gart, J.J.: On the ordering of contingency tables for significance tables. Technometrics 13: 910-911, 1971.

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Gart, J.J.: Interaction tests for 2 x s x t contingency tables. Biometrika 59: in press, 1972.

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Gart, J.J. and Thomas, D.G.: Numerical results on approximate confidence limits for the odds ratio. J. Roy. Stat. Soc. B, 34: in press, 1972.

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Thomas, D.G.: Exact confidence limits for the odds ratio in a 2 x 2 table. Applied Statistics 20: 105-110, 1971.

Thomas, D.G.: Tests of fit for a one-hit vs. two-hit curve. Applied Statistics 21: in press, 1972.

Thomas, D.G. and Gart, J.J.: The small sample performance of some estimators of the truncated binomial distribution. J. Amer. Statist. Assoc.: 66: 169-177, 1971.

Serial No. NCI-4268

1. Biometry Branch, OASDD, Etiology
2. Special Cancer Survey Section
3. Bethesda, Maryland

NIH

Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Third National Cancer Survey

Previous Serial Number: Same

Principal Investigators: Harvey Geller and Sidney J. Cutler

Other Investigators: Roger Connelly, Daniel Cramer, Susan S. Devesa, Kenneth Hurst, James Larson, Winifred M. Mendez, Constance Percy, Joseph Scotto, Theodore Weiss

Cooperating Units: University of Alabama School of Medicine
Georgia Regional Medical Program
State of Colorado Department of Health
University of Texas Southwestern Medical School
Michigan Cancer Foundation
University of Iowa
Mayo Clinic
University of Minnesota, Department of Epidemiology, School of Public Health
University of Pittsburgh, School of Public Health
Puerto Rico Commonwealth Health Department
State of California, Department of Public Health
Iowa State University
Epidemiologic Pathology Unit, NCI
Electronic Data Processing Staff, BB, NCI

Man Years:

Total:	11.5
Professional:	8.0
Other:	3.5

Project Description

Objectives:

To prepare a complete register of all new cancer cases diagnosed in the resident population of seven standard metropolitan areas, two states, and the Commonwealth of Puerto Rico during the period 1969-1971, plus all other cancer cases seen by doctors or hospitals in those areas; to obtain demographic data and a description of the tumor for each patient; and to study certain economic, social, and other aspects of the disease in a sample of patients.

Extensive use is being made of computer capabilities in the processing of the survey documents. Each document is processed separately, with the computer tagging and combining all the records for an individual patient. The computer is conducting a large number of edit checks--consistency among documents on the same patient, as well as among items on the same document. The sample selection is also a computer operation with the printing of the appropriate labels included in the operation. The data processing time has been reduced to a minimum. Within five weeks after a record is received at NCI, the data are converted on to computer tapes, edit runs are completed, and printouts of error reports and updates of survey control reports are mailed back to the field offices.

Methods Employed:

In each survey area, NCI has contracted with local organizations to hire field staff and gather data. Data are being requested from doctors and obtained from hospital records and death certificates. With approval of their doctors, a sample of cancer patients is being interviewed. Local staffs do the preliminary editing and coding of data, and are responsible for disposition of the original records at the close of the survey. Institute staff in Bethesda, Maryland, complete the editing and coding of data and convert it to magnetic tape for use in preparing detailed reports through electronic computers.

Basic information is being collected on every new cancer case, except certain skin cancers, during the three-year period. This includes race, age, place and date of birth, sex, and marital status. Medical information includes the primary site and histologic type of tumor, date and method of diagnosis. Information available from any existing cancer register or continuing survey in a survey area is being utilized to avoid duplication of effort. All reports are treated as strictly confidential.

Death certificates mentioning cancer are obtained from the appropriate vital statistics office. Local survey staff or hospital personnel abstract information about cancer patients from hospital records.

Trained interviewers, after receiving written permission from attending doctors, obtain from a selected sample of patients information on the amount of disability incurred, time lost from productive activity, cost of treatment, and sources of payment. Questions are also asked regarding religion, number of children, socioeconomic status, and personal habits such as smoking and drinking. Hospital records on this selected population are reviewed for more detailed information on cost of hospitalization, methods of meeting this cost, treatment for the cancer, and the extent of the disease at time of diagnosis.

The main products of this survey will be a detailed analysis of cancer incidence and prevalence rates, the extent of the disease at time of diagnosis, the medical treatment, and the hospital costs. There will also be a large number of special reports either by the survey staff or others with

an interest in more detailed analysis of the data being collected. Reports for each individual survey area will also be prepared.

Workshops are conducted periodically to resolve problems which the supervisors have encountered. The format of round table discussions on specific questions and informal discussions among the supervisors have proved very effective in carrying out the instructional and communicative objectives of these workshops.

Editing the data within a document and among documents on the same patient is accomplished by extensive use of the computer. Data are accessed on a weekly cycle and collated into the master file. Each item is reviewed to insure that it is within set limits and that the coded information is a valid code. Other edits include determination of consistency or validity between items on the same abstract form. The computer also checks for consistency in data among the various forms on the same patient. There is a feedback to the survey areas of any invalid, inconsistent, or questionable data. The survey field offices research the problems and correct the data stored in the computer tapes. The data are reviewed periodically to insure that corrective action has been taken by the survey field offices.

Because of the limited staff available, some of the quality control procedures have been modified. The reinterview program was eliminated because of the problems in obtaining the reinterviews, and the lack of feedback either to the survey field office staff or the NCI. The reabstract program has also been modified by eliminating the reabstracting of a random sample. At the present time, reabstracting is accomplished at the same time as the initial abstract, and comparisons are made at the hospital with the records available.

A special skin cancer survey was started September 1, 1971, which collected data on cases diagnosed through February 29, 1972. Only four of the survey areas--Iowa, Dallas, Minneapolis, and San Francisco--are participating in this special study. Special effort was made to contact all physicians who would see or treat skin cancer patients to obtain their cooperation. Pathology laboratory files will supply data on cases that were histologically diagnosed. In addition, abstractors will complete separate forms for skin cancer patients found during their normal visits to participating hospitals.

This special study is being handled as a separate operation. A separate report will be published presenting the skin cancer data as soon as the data are tabulated and analyzed.

Major Findings:

A preliminary report based on the 1969 incidence data was distributed to the National Cancer Advisory Council in October, 1971. The report showed that 61,410 cancers were diagnosed in the survey areas (excluding the non-Denver portion of Colorado, and Puerto Rico) during 1969. Since the survey areas include ten percent of the total United States population, it is estimated that approximately 610,000 new cancers were diagnosed in that year. The report further showed that when the rates for sex and race are adjusted to

the age distribution of the United States in 1970, the incidence rate for the black population (338 per 100,000) is substantially higher than for the white population (311 per 100,000). The sites with markedly higher rates among black males are the prostate and the esophagus. Among females, the black females have higher rates for both invasive and in situ carcinoma of the cervix uteri, while the white females had higher rates for cancer of the breast and corpus uteri.

While the areas covered by the present survey are not exactly the same as the areas covered in the survey of 1947, a number of trends are worthy of note. Adjusting the rates to the United States 1950 population, the incidence rates among males increased from 280 in 1947 to 304 in 1969, while the rates among females decreased from 294 to 256 (excluding carcinoma of the cervix uteri) for the same time periods. The increase in the male rate is due largely to substantial increase in the incidence of cancers of the lung and prostate, and a somewhat lesser increase in incidence of colon cancer. These increases more than counterbalanced the drop in incidence of gastric and rectal cancers. The decrease in the female rate is the result of a drop in the incidence of cancers of the cervix uteri (invasive), stomach, and rectum.

The data also showed that when the survey areas were divided into three geographic regions--North, South, and West--cancer of the lung, prostate, digestive tract, breast and uterus demonstrated geographic differences.

Significance of the Program to the Institute:

Registration of all cancer cases in a defined population is the only way to acquire reliable data on cancer incidence rates. The rates themselves are indicators of the significance of cancers in the various sites or of different types. This data bank would also provide a valuable source for epidemiologic investigations by identifying population groups that have a high or a low incidence of cancers of specific sites. Some of the factors that would be studied include residency, occupation, place of birth, use of contraceptive devices, and the use of tobacco and alcohol.

Other products of the survey that will be important to the mission of the National Cancer Institute will be studies on the delivery of medical care to a large and representative sample of cancer patients, treatment provided the cancer patient, extent of the disease at the time of diagnosis, direct and indirect costs of cancer, and the extent and duration of disability from cancer.

The survey will produce unique data which can be used by other investigators as a starting point for more intensive investigations. One area of special interest is rare tumors. Because of the low numbers of these tumors, epidemiologists feel that investigations will yield significant results and possible etiologic leads.

The mass of data accumulated by the survey will afford a quick query as to what is occurring with regard to specific forms of cancer. For example, the survey was able to obtain a tabulation of the number of vaginal cancers occurring in women under age 20 for those diagnosed in 1969 when it was reported that daughters of women who received hormonal therapy during pregnancy had an increased risk for vaginal cancer. It will also flag those diagnosed in 1970 and 1971, thereby supplying up-to-date data from a large population on the change, if any, in this cancer site in this population.

Proposed Course of the Project:

During the early part of the survey, as was expected from a project of its size, many modifications were necessary in the operating procedures. As questions arose and the data that were being received at NCI were evaluated, changes were made to insure complete coverage of the population, uniformity of the data being reported, and accuracy of abstracted data and coded information. Most of the modifications have been made, and very little further changes will be made. The computer system is also relatively stabilized, except for elimination of specific checks for items which would not be used in the analysis or where the completeness or accuracy is doubtful. The computer printouts have been expanded to provide more feedback of data to the survey areas. This not only reduces the amount of work that has to be done at the field offices in editing and records search, but will contribute to a more complete coverage of the survey population.

Now that there is a data bank of information relating to 1969 incidence, some investigations of this data will be accomplished. These investigations will have a number of benefits. They will provide some evaluation of the data accumulated as to its accuracy and completeness. They will also give indications of the usefulness of the data, and point up special studies which should or could be undertaken. These investigations will also be useful in planning the analysis of the complete survey.

Most of the effort, during the first half of 1971, was pointed toward completing the reporting of the 1969 cases. This meant that all survey areas set a high priority for operations which were required to complete this phase of the survey. All methods of case finding, such as searching the pathology registers, autopsy reports, radiotherapy rosters, cancer registries, and hospital diagnostic files, were used to insure complete reporting of all cancer cases. Due dates for the finishing of specific operations were set so that all the data would be available in time for edit and return to the field offices for correction at a specific date. Another date was set when all corrections to records of patients with active disease in 1969 were to be received at NCI. As a result of this effort, the data on 1969 cases were ready for final tabulation by the end of July. Concurrent with the effort to complete reporting, the computer programs needed to tabulate the data and compute the various rates (such as crude rates, age-specific rates, and age-adjusted rates using the two different populations as the Standard, 1950 and 1970 total United States population) were being developed and tested. As a result, all specified tabulations were available for evaluation by the

end of August. The text material was written and final copy sent to the printer in early September. Adhering to the schedule resulted in a publication being available for distribution to the National Cancer Advisory Council at its meeting the first week in October, 1971.

In order to meet the demand for data by the news media, cancer investigators, and others, an abridged version of the report was produced which contained the text material and the important summary tables. This abridged version was also used by the survey field offices for distribution to those cooperating in the survey. Tabulations were also generated for each survey area, presenting data for that area. In addition, a tabulation of cases by hospital showing the experience of that hospital was produced for distribution to the hospitals by the survey field offices.

With the 1969 incidence report completed, effort will be concentrated on the production of a report on medical care and medical economics of a sample of patients diagnosed with cancer in 1969. This will require all the survey field offices participating in the sample study to identify all hospitalizations of the sample patients occurring during the survey period. Medical supplements will have to be completed, describing the surgical procedures and indicating whether the patient also received chemotherapy or radiotherapy during these hospitalizations.

Financial offices of each hospital will provide data on charges and payments for every cancer-related hospitalization. The payment data will indicate source of payment such as Medicare, Medicaid, Blue Cross, other insurance, and the amount paid directly by the patient. Where possible, data will also be obtained regarding the portion of the charges that are written off as a result of discounts given Blue Cross or Medicare.

Another facet of the sample study is the determination of the stage of the disease at the time of diagnosis. The progression of the disease at the time of diagnosis will be specified using the End Results criteria and code for extent of disease. This code is site-specific and uses all of the information available regarding such factors as size of lesion, extension into adjacent tissue, nodal involvement, and remote metastases. In the determination of the extent of disease, all information available up to four months after diagnosis is used. This includes information at time of surgery, pathological diagnosis, clinical findings, and autopsy data.

It is anticipated that the survey operation will start phasing out during fiscal year 1973. Abstracting of hospital records should be completed by the end of 1972. During the first six months of 1973, deaths occurring in 1972 will be abstracted. The year 1973 will also be used for abstracting supplemental data on 1972 hospitalizations for sample cases with a first report to the survey in 1970 or 1971. The patient interviews still being done in San Francisco, Detroit, Birmingham, Dallas and Colorado survey areas will also need this time to complete the work on patients selected for the sample late in the survey.

Following this time schedule, all data for incidence and prevalence should be available for final tabulation late in 1973. Preparing the material for publication and writing the text material to accompany the tables will most likely mean that a final report will be available during the last half of 1974. Once this report has been published and distributed, staff will engage in a more detailed analysis of the survey data.

Honors and Awards:

None

Publications:

Preliminary Report, Third National Cancer Survey, 1969 Incidence

Serial No. NCI-4269
1. Biometry Branch, OASDD, Etiology
2. Automatic Data Processing Management Section (ADPMS)
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Data and Information Processing Consultation and Service

Previous Serial Number: None

Principal Investigator: Theodore Weiss

Other Investigators: Terence D.C. Kuch, James E. Larson, Frank W. McKay, Norman Oslik, James M. Stump

Cooperating Units: None

Man Years:
Total: 7.0
Professional: 6.0
Other: 1.0

Project Description

The Automatic Data Processing Management Section provides consultation and service to the scientific and administrative staff of the Etiology Area of the Institute. It has expertise in applying data and information processing technologies to epidemiologic, laboratory, and clinical research projects, program planning and appraisal activities, and administrative management operations.

Objective:

To facilitate the research effort through the appropriate application of computer science and information technology, and through minimizing the mechanical and logistical constraints upon investigators arising from activities which involve data and information collection, conversion, purification, computation, storage, retrieval, analysis, and dissemination.

Methods Employed:

Consultation and support is provided to research projects in three disciplinary areas:

Planning and Appraisal: participation in the planning aspects of research projects which have data and information processing activities; conducting studies and disseminating information on

the feasibility of using computer-related techniques, such as communication networks, computer-related proprietary software packages, and computer-related equipment; and developing standards of quality and performance of data and information processing products and services provided by the ADPMS, commercial ADP contractors, and research contractors;

Information Systems Development and Statistical Computing: providing technical support in the form of systems analysis, systems design, and computer programming services using digital computers, scientific computers with analog-digital converters, and related electronic instrumentation; and,

Operational Monitoring and Control: converting raw data into forms appropriate for computer processing, the day-to-day coordination, liaison, and operation of data processing systems, and the operation of such equipment as the ADPMS IBM 360/20 Remote Job Entry Terminal.

Major Findings:

Office of the Associate Scientific Director for Program

A Program Monitoring System is currently being developed under the direction of the Associate Scientific Director for Program to facilitate Etiology Area-wide program planning and contract monitoring. A pilot system has been implemented in the Carcinogenesis Area to determine the feasibility, acceptability, and cost of such a system as well as to ascertain the optimum points of data entry and control.

Viral Oncology Area

Consultation has been provided the Program Analysis and Communications Office, Special Virus Cancer Program regarding the information systems aspects of biological resources and logistics management.

Carcinogenesis Area

The Program and Data Analysis Unit in the Carcinogenesis Area is receiving support from the ADPMS through the development and implementation of a document storage and retrieval subsystem of the Carcinogenesis Bioassay Data System. This has involved conducting a comprehensive study of document storage and retrieval techniques, the development of detailed system specifications, the recommendation of a specific microfilming system, the preparation of a comprehensive RFP, and participation in the selection of a contractor to provide this service. In addition, the ADPMS is coordinating a variety of other Carcinogenesis Area data processing activities.

Demography Area

There have been a number of significant accomplishments in the Demography Area this fiscal year in the area of data processing and statistical computing:

A generalized table-making and data retrieval system, Generalized Reporting Program (GRP), was installed and made available, which allows investigators access to their computerized data through remote terminals (WYLBUR), by entering a few simple instructions on these terminals;

A study is being conducted by an ad hoc committee of representatives from all Sections within the Area, of existing research projects and of those projected over the next five years, to ascertain the ADP software, hardware, and staffing needs of the Demography Area, the approximate cost of these activities, and the level of contractor versus in-house sources of ADP support;

A more comprehensive system of Program and budget control over computer programming and machine costs has been implemented. Investigators are receiving detailed monthly reports of all costs incurred by them for these functions, whether provided by data processing contractors, the Division of Computer Research and Technology, NIH, or commercial time-sharing facility; and

A general software contract for biomedical information systems support was implemented for the Etiology Area through the ADP Management Section. This is a task-oriented Basic Ordering Agreement and may be used by any segment to obtain systems and programming services.

The ADP Management Section is providing the Demography Area with three prime areas of service; large-scale data processing systems, prepackaged software for investigators' own use, and statistical programming. This support has encompassed ten research projects:

Computer programming support was provided for three Epidemiology Branch studies. Statistical tables, graphs, and maps were produced for the Study of Cancer Mortality in Indians and the Study of Cancer Mortality by County in Colorado. Several computer programs were modified to reflect changes in the Veterinary Medical Data Program (VMDP). The monthly data processing activities of the VMDP at a commercial data processing contractor and the Division of Computer Research and Technology are monitored by the ADPMS;

The Norwegian Migrant Study, the Study of Cancer Among Retired Railroad Employees, and the Japanese Migrant Studies received computer programming and operational support from the ADPMS.

Several computer programs were written in conjunction with the preparation of the End Results in Cancer Report 4. A file-independent computer system which will calculate frequencies and age-adjusted and age-specific incidence rates from user supplied parameters is under development primarily for the End Results Group, but has application elsewhere in the Demography Area;

Computer programming support and statistical consultation has been provided to the Clinical Trials research program;

A comprehensive data processing system encompassing clinical, laboratory, and epidemiologic data, portions of which are now operational in Hawaii, is being developed for the Japan-Hawaii Cancer Study;

The Special Cancer Survey Section is receiving extensive technical ADP support to operate and maintain the Third National Cancer Survey's data processing system, and to generate reports for the analysis and publication of Survey data. This included the technical direction of all activities involved in the conversion of data from some 340,000 source documents to magnetic tape, the operation and maintenance of the data processing system, and the design and implementation of new computer programs and subsystems to meet specific operational and analytical needs. This effort included the development of a special system to produce 133 separate tables, in camera-ready form, for the Preliminary Report of 1969 Incidence.

Administrative Office

Each month the ADP Management Section updates its computer files containing contract commitments summary data for the Etiology Area. This is a fairly extensive operation because of the amount of contract activity and the dual appropriation. Monthly the Section produces the Contract Commitment Report for the Administrative Office. The production of this report by the ADPMS is expected to end when the Program Monitoring System becomes operational.

Office of the Associate Director for Program Planning and Evaluation, NIH

A limited amount of consultation was provided the Office of the Associate Director for Program Planning and Evaluation, NIH, regarding the application of our generalized geographic mapping and histogram computer programs. This Office is trying to determine whether our computer programs can be used to reduce the effort and time required to develop visual aids which depict funding levels of total federal and NIH supported research and related activities in each of the States. These same techniques should be applicable to Etiology Area reporting activities.

Significance to the Program of the Institute:

The systematic capture, organization, and display of complex and diverse data is of considerable importance in the planning, conduct, and management of the research efforts. As multidisciplinary and collaborative activities increase in scope and complexity, the problems of linking, manipulating, analyzing, and communicating large quantities of data and information become unmanageable without the assistance of computer-related technology.

Proposed Course of the Project:

Consultation and technical support will continue to be provided to Etiology Area research activities. The level of support is expected to increase as the National Cancer Plan is implemented.

Honors and Awards

Weiss, T.: Member, Contractor Selection Committee, Office of Program Planning and Analysis, Office of the Director, NCI, Logistics Support in the Preparation of the National Cancer Plan; Member, Grant Application Review Committee, NHLI Special Center of Research, Department of Preventive Medicine, University of Wisconsin Medical School; Member, Grant Application Review Committee, NHLI Special Center of Research, Department of Surgery, Boston University School of Medicine.

Publications

Kuch, T.D.C.: Professionalism. National Conference of the Association of Computer Programmers and Analysts, 1971. (Invited Paper).

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
FISCAL YEAR 1972

Office of the Chief

HEALTH INSURANCE PLAN OF GREATER NEW YORK (69-88)

Title: Evaluation of Periodic Breast Cancer Screening by Mammography

Contractor's Project Director: Mr. Sam Shapiro

Project Officer (NCI): Mr. William Haenszel

Objectives: A. To determine whether a simple screening technique (x-ray mammography) can diagnose breast cancer early, and

B. To determine if the very early diagnosis of breast cancer is really useful in improving long-term survival.

Methods Employed: This is a combination statistical-epidemiological-medical study in which a study population receiving mammography at annual intervals and a control group of 30,000 women each are being routinely followed for breast cancer experience through internal Health Insurance Plan files and through review of death certificates. Details of the techniques are given in a paper describing the methodology and initial findings of this study entitled, "Evaluation of Periodic Breast Cancer Screening with Mammography" by Sam Shapiro, Philip Strax, and Louis Venet, JAMA 195: 731-738, 1966.

During one reexamination cycle 40 cc of blood was taken from women in the study population and stored.

Major Findings: Findings to date strongly suggest the usefulness of annual screening which includes mammography. Of 132 breast cancers diagnosed to date among women in the study population, 44 would have been missed in the screening examination if mammography had not been used. The proportion of breast cancers detected on screening with no evidence of nodal involvement is high -- (more than) 70 percent, compared with 46 percent for women in the control population. Preliminary data on mortality point to the prognostic advantage of an early diagnosis. A total of 49 deaths due to breast cancer have occurred to date in the study population, compared with 70 deaths in the control population.

Significance to NCI Program & Bio-Medical Research: Early diagnostic procedures may be of use in the early treatment and potential cure of cancer patients. If it develops that mammography is such a technique, it then could be extended to other populations and would then have the effect of reducing the mortality from breast cancer within the United States.

Proposed Course of Project: The screening part of the study is complete and the emphasis from now on will be on follow-up. The women will be followed for as long a time period as necessary to demonstrate whether the technique has any usefulness as any early diagnostic device or whether its usefulness is limited. The duration of the study is contingent upon the answers to the questions implied by the objectives.

Supplemental lines of inquiry may be suggested by the Breast Cancer Task Force. Proposals for use of the stored blood plasma are being developed.

Date Current Contract Initiated: 11-1-62

Current Annual Level: $196,300

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1972

Office of the Chief

JOHNS HOPKINS UNIVERSITY (71-2422)

Title: Epidemiological Study of Rare Sites of Cancer

Contractor's Project Director: Dr. Abraham M. Lilienfeld

Project Officer (NCI): Mr. William Haenszel

Objectives: To elucidate the causal factors in the development of selected rare cancers in patient groups which are large enough to make the findings valid and reliable. The rare sites to be studied will be liver, gallbladder, islet cell tumors of the pancreas, renal pelvis, nasopharynx, adrenal gland, testis, penis, scrotum, vagina, vulva, and male breast.

Methods Employed: The investigation will take the form of a case-control study. The appropriate cases and matched controls will be assessed, interviews will be obtained and their medical records checked. A total of five areas are included, two of which are also part of the Third National Cancer Survey (Buffalo, Detroit, Miami, Minneapolis-St. Paul, and New York City).

Major Findings: This contract is too new for any major findings. However, previously found information is available on the neoplasms, and the relationship between cancer in some rare sites and its causal factor has been fairly well established.

Significance to NCI Program & Bio-Medical Research: By identifying all known malignant neoplasms soon after the initial diagnosis, The Third National Cancer Survey could permit patient interviews while many patients are still in reasonably good health and able to participate in the study.

Proposed Course of Project: Known carcinogens account for a relatively small proportion of the incident cases, therefore, it is desirable to search for additional factors associated with rare neoplasms. The questionnaire format will integrate the proposed study with several other study programs under the Third National Cancer Study.

Date Contract Initiated: 6-28-71

Current Annual Level: $40,000

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
FISCAL YEAR 1972

Office of the Chief
(With Colon Cancer Segment, Carcinogenesis)

LOUISIANA STATE UNIVERSITY MEDICAL CENTER (NIH-71-2324)

Title: Premalignant Lesions in the Large Intestine

Contractor's Project Director: Professor Jack P. Strong

Project Officer (NCI): Dr. John W. Berg

Objective: To obtain data on the prevalence of various kinds of epithelial intestinal tumors in white and black Americans corresponding to data obtained or being obtained from other populations of epidemiologic interest.

Methods Employed: Tumors are identified by a careful protocol study of bowel specimens obtained at autopsy.

Major Findings: A preliminary study indicated that the prevalence of small tumors is quite different in populations of low and high risk for colon cancer. The number of cases is not yet sufficient to provide usable estimates for prevalence in population subgroups and for specific anatomic regions of the bowel.

Significance to NCI Program and Biomedical Research: It is hoped to identify those epithelial lesions which are epidemiologically related to colon cancer. This will not only provide clues on the pathogenesis but identify individuals at high risk for colon cancer and provide a signal of exposure closer in time to the contact of patient and environmental factors.

Proposed Course: It is expected that at least another year will be required to collect enough samples to give reliable estimates of lesion frequencies and distributions in all age-sex-racial groups. Meanwhile, a study on DNA content of the cells of the various lesions is being started in the expectation that this may clarify classification of lesions difficult to classify (which are frequent).

Date Contract Initiated: June 1, 1971

Current Annual Level: $27,500

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1972

Office of the Chief

NORWEGIAN PUBLIC HEALTH SERVICE (64-499)
UNIVERSITY OF MINNESOTA (66-919)

Title: Incidence, Prevalence and Mortality from Cancer in Selected Migrant Populations (Norwegian)

Contractor's Project Director: Dr. Einar Pedersen (64-499)
Dr. Leonard Schuman (66-919)

Project Officer (NCI): Mr. William Haenszel

Objectives: To determine the reason for difference in cancer incidence and mortality in sedente populations and migrant populations of the same ethnic and genetic composition.

Methods Employed: Standard epidemiologic-demographic methods including prospective observations on defined cohorts and case-control studies.

Major Findings: The introduction of personal identification numbers in Norway has enormously simplified the surveillance of all defined study cohorts, including factory employees. During the past year, Dr. Pedersen was asked to trace the cancer experience of employees at the Falconbridge nickel factory. Given the personal identification numbers, the type and duration of work at the plant, etc., he was able to establish with very little effort the distribution of cancer cases for all employees with a work history at the plant since 1946. While follow-up and analysis is not completed, results in hand demonstrate an excess lung cancer risk in this population. While Norway is not heavily industrialized, there are a variety of employee populations in which studies of occupational carcinogenesis could easily be carried out. Since the research contract for the Norwegian migrant studies has supported installation of data systems to establish record linkages for the Norwegian cohorts, an obvious resource for studies of employee populations and occupational carcinogenesis has been established as a by-product.

A paper summarizing the results of case-control studies of gastrointestinal cancer in Norway and Minnesota (for persons of Norwegian descent) is nearing completion. The findings with respect to associations with diet history and other characteristics are at best only suggestive, but will have value when collated with the findings for other migrant groups, in particular Japanese migrants to the United States.

Breast cancer studies in Norway and among Norwegian migrants have been deferred until the results of a 9-year follow-up of 80,000 women between the ages 25 and 65 whose examination included breast palpation by a physician and collection of a reproductive history, have been completed.

Significance to NCI Program & Bio-Medical Research: This is one of a group of migrant studies to determine those elements in the incidence and mortality from cancer which may be related to environment and those which may be related to genetic factors. Isolation of environmental elements may be useful in cancer prevention within the United States.

Proposed Course of Project: The next contract year will represent a period of consolidation for the epidemiological phase represented by the general population and sib cohort studies. Analysis of the initial 6 years follow-up must precede the definition of studies directed to specific sites and specific hypotheses. The level of support for staff concerned with data management should continue unchanged. The major area of new work in the coming year will be the collection of additional pathology protocols for selected sites to expand on the incidence data. Thyroid is a promising site for this effort. Great differences between coastal and inland areas in Norway for thyroid cancer and efforts to pinpoint pathological entities responsible for this difference should have high priority.

The University of Minnesota has defined a cohort of approximately 20,000 policyholders in an insurance company that has a high proportion of persons of Norwegian descent and will monitor the group prospectively for mortality data. The contractor has also carried out case-control interviews for gastrointestinal cancer among persons of Norwegian descent in Minnesota and is prepared to extend field work to other sites at a later date. All phases of the cohort and case-control studies in Minnesota are closely coordinated with the ongoing work in Norway. One investigator from the Cancer Registry of Norway is in residence almost continuously at the University of Minnesota to assure close liaison.

Date Contract Initiated:	March	1964 (64-499)
	June	1966 (66-919)
Current Annual Level:	$31,863	
	$13,278	

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1972

Office of the Chief

RESEARCH CORPORATION OF UNIVERSITY OF HAWAII (71-2208)

Title: Demographic Cancer Research and Training Program in Hawaii

Contractor's Project Director: Dr. Richard K. C. Lee

Project Officer (NCI): Mr. William Haenszel

Objectives: The objective of this project is to plan and develop a Demographic Cancer Research and Training Program in Hawaii. This Program will form a basis for a future comprehensive cancer research center.

Methods Employed: Standard methods of planning, programming and budgeting, as well as personnel recruitment and management are employed.

Major Findings: The work is based on current plans as described in a progress report prepared under this contract which outlines the goals of the Demographic Cancer Research and Training Program and the specific objectives for both the research and training aspects of the program. The proposed activities for the first phase of this Program include Resource Development, which will provide a population-based cancer data and computation system for collection of statewide data, support for a statewide tumor registry and work on development of new cohorts of racial groups other than Japanese. Activities will also include a Training Program with both University components and field resources available in Hawaii for training.

Significance to NCI Program and Biomedical Research: The development of a Demographic Cancer Research and Training Program in Hawaii is directly related to the Demography Program plans for development of population-based cancer registries as recommended by the Ad Hoc Advisory Committee on Population-based Epidemiology Research Centers. It is also directly related to development of comprehensive clinical cancer research centers as defined by Congress in The National Cancer Act of 1971. The new program will serve as a cancer incidence reporting system, a data resource, an epidemiological research effort and as a much needed training base. Eventual inclusion of the Demographic Program into a comprehensive clinical cancer research center will further increase the usefulness and productivity of the demographic work and will strengthen the total training program.

Proposed Course of the Project: This project should continue for one or two more years at which time the Demographic Program will become operational at the University of Hawaii.

Date Contract Initiated: June 1971

Current Annual Level: $47,292

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1972

Office of the Chief

TOHOKU UNIVERSITY SCHOOL OF MEDICINE (64-10)
UNIVERSITY OF HAWAII (63-558)
CALIFORNIA DEPARTMENT OF PUBLIC HEALTH (64-873)
KUAKINI HOSPITAL AND HOME (71-2170)
AICHI CANCER CENTER RESEARCH INSTITUTE (72-3213) (REPLACES TOHOKU UNIVERSITY)

Title: Study of Cancer Among Japanese Migrants

Contractor's Project Director: Professor Mitsuo Segi (64-10)
Dr. Richard K. C. Lee (63-558)
Dr. John E. Dunn, Jr. (64-873)
Dr. Grant N. Stemmermann (71-2170)
Professor Mitsuo Segi (72-3213)

Project Officer (NCI): Mr. William Haenszel

Objectives: The purpose of these contracts is to collect data bearing on the reasons for the differences in cancer incidence between Japanese on the home islands and the Japanese migrant populations in Hawaii and California. The objective is to sort out those aspects of common cancers which may be genetically involved and those which may derive from aspects of the environment or some mixture of the two.

Methods Employed: Standard demographic techniques are employed, basically using a system of diagnosed cases and matched controls. In addition, data have been collected from samples of Japanese households. The matched case-control technique is particularly useful in this kind of study. Pathology protocols are being completed for cases in the study series for several sites (stomach, colon, rectum) and considerable emphasis is being placed on the correlation of the epidemiological and pathology findings. In preparation for later case-control studies of prostate cancer, latent prostate cancer is being studied in 5 groups of patients.

Major Findings: The cancer risks for Japanese migrants differ from those observed in Japan; the migrant risks are higher for lung, colon, rectum, prostate and lower for stomach while little change has been observed for breast. To date, the potential information exists tending to incriminate changes in dietary habits and in smoking habits for the changes in stomach cancer and lung cancer in the Japanese populations. Latent prostate cancer is almost as common in Japanese as in Americans though Japanese have little clinical cancer of this site. Japanese migrants to Hawaii have an intermediate position as to prevalence but show a complete shift in histology from "Japanese" to "American" type.

Significance to NCI Program & Bio-Medical Research: If the studies in these migrant populations elucidate the environmental factors involved in the incidence of certain forms of cancer, and indicate those that are predominantly environmental and those which are predominantly genetic, then it may be possible to apply these findings to the United States population for purposes of cancer prevention and cancer control.

Proposed Course of the Project: The contract with Aichi Cancer Center Research Institute has been initiated to continue Dr. Segi's work in Japan after his move from Tohoku University. The contract with Tohoku University has been terminated. A new NCI contract has been started with Kuakini Hospital and Home, separate from the NHLI contract, to assure proper support and direction of the important clinical and pathological cancer research being performed at Kuakini.

Date Contract Initiated:	July	1964 (64-10)
	April	1963 (63-558)
	May	1964 (64-873)
	June	1971 (71-2170)
	February	1972 (72-3213)
	June	1971 (71-2208)

Current Annual Level:	$95,549	(64-10)
	$57,235	(63-558)
	$50,000	(64-873)
	$450,000	(71-2170)
	$91,384	(72-3213)
	$42,304	(71-2208)

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1972

Office of the Chief

UNIVERSIDAD DEL VALLE -- FUNDACION PARA LA EDUCACION SUPERIOR (PH43-66-907)

Title: Epidemiology-pathology studies of cancer in Colombia

Contractor's Project Director: Dr. Carlos Cuello

Project Officers (NCI): Mr. William Haenszel, Dr. John Berg

Objectives: To determine site-specific cancer incidence in Cali and other cities in Colombia and to correlate the morbidity data with pathology findings.

Methods Employed: The cancer morbidity surveys in Cali will be continued with emphasis on variation by socioeconomic class and place of birth. Internal migration within Colombia is of such magnitude that two-thirds of the residents of Cali were born elsewhere. Cancer morbidity surveys on a limited scale are being undertaken in Cartegena and Medellin, cities thought to have greatly different site-specific incidence.

Studies of autopsy materials and surgical specimens are being conducted in Cali to define criteria which differentiate high-risk and low-risk populations. In Cali an organ bank of tissues from persons dying from accidents and acute diseases is being utilized for study of occult tumors and malignant precursors with emphasis on stomach, colon and cervix. The organ bank findings also emphasize the distribution by place of birth and are used to complement the incidence data from the morbidity surveys. Similar pathologic material is being collected in New Orleans and Hawaii.

Major Findings: The findings from the Cali Morbidity Survey were included in the initial UICC publication, Cancer Incidence in Five Continents, and more comprehensive data were included in the second edition published in 1970.

Cancer premalignant leisons of the stomach have received special attention and the findings from the Cali cancer register and organ bank have been analyzed. This work has helped to clarify the possible role of intestinal metaplasia of the gastric mucosa as a premalignant leison and contributed to the development of some hypotheses on pathogenesis. It is worth noting that these findings display many points of consistency with observations made on Japanese migrants.

The Colombia group has continued pursuit of work in the pathology and epidemiology of thyroid carcinoma, which began with comparisons of the Cali and Medellin experience to international comparative studies. Much of this work has involved comparisons of incidence and histologic type distribution in Cali and Connecticut. The work of the Cali and other investigators point to papillary, follicular and anaplastic carcinomas of the thyroid as separate entities.

Review of the series of colons in the Cali organ bank now indicates that the prevalence of intestinal polyps in the population of Cali (at low risk to cancer of the colon and rectum) differs in many important respects -- sex and age distribution, location, multiplicity and atypia -- when compared to published, but incomplete and fragmentary findings on populations at high risk to colon cancer. Attention has been given to standardization of procedures so that comparable data may be assembled in other localities. The findings for Cali are being prepared for publication and they will serve as a useful benchmark for reference, but interpretation and assessment of their significance will depend on the production of comparable data for other populations in the United States and other countries. This activity will be an important component of Dr. Correa's work in the United States.

Significance to NCI Program & Bio-Medical Research: This contract is producing significant data for comparative studies in the United States and elsewhere, with emphasis on those areas providing the greatest contrasts in risks for specific cancer sites.

Proposed Course of Project: Collection of data on gradient of cancer risks within Colombia by socioeconomic class, place of birth and current residence, etc. will be continued. Much effort will need to be placed on refinement of pathology criteria and their test as discriminants between high- and low-risk areas. Priority in study of the pathology materials will be given to stomach, colon, cervix, and thyroid. Emphasis in the coming year will be on clinical and experimental studies of the genesis of intestinal metaplasia in Colombia.

Date Contract Initiated: June 1966

Current Annual Level: $41,260

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
FISCAL YEAR 1972

Office of the Chief
(With Colon Cancer Segment, Carcinogenesis)

UNIVERSITY OF CALIFORNIA, LOS ANGELES (NIH-NCI-E-72-3209)

Title: Fecal Flora Studies

Contractor's Project Directors: Dr. S. M. Finegold
Dr. H. R. Attebury

Project Officer (NCI): Dr. S. J. Silverman

Objectives: Perform a comparative study of the fecal flora of Japanese living in the Los Angeles Area. The subjects, to be selected with the aid of epidemiologists of the State of California Department of Health will include individuals on a traditional Japanese diet, on a western diet, and a small number with intestinal polyps or early proven carcinoma of the bowel, as well as individuals with a positive test for CEA but without demonstrable carcinoma.

Major Findings: Negotiations for this contract have just been completed.

Significance to Biomedical Research and the Program of the Institute: It is expected that the findings will clarify some of the epidemiological factors leading to cancer of the large intestine. Occurrence of carcinoma with increased frequency in migrant population will be correlated with changes in their life habits.

Proposed Course: Feces will be examined from individuals selected because of the presence of polyps or early carcinoma of the colon as well as from healthy individuals selected on the basis of their diet and socio-economic characteristics. This study will rely heavily on the use of selective media and procedures to isolate and identify the bacteria present. By the use of selective media, it should be possible to detect organisms that are inhibited in the presence of other bacteria or are present in small numbers. It may thus be possible to detect "indicator organisms" present in cancerous or precancerous conditions.

Date Contract Initiated: March 2, 1972

Current Annual Level: $71,930

Publications: None

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1972

Office of the Chief

U.S. RAILROAD RETIREMENT BOARD (FS-50)

Title: A Study of Cancer Mortality among Employees in the Railroad Industry.

Contractor's Project Director: Robert McNab

Project Officer (NCI): Benjamin E. Carroll

Objectives: To determine mortality rates from cancer among active and retired railroad employees and to compare cancer mortality among employees having various types of exposure due to occupation and to the use of Diesel locomotives by employers.

Methods Employed: Cohorts of active and retired employees were selected from RRB records as of Dec. 31, 1951, totalling 1,100,000 persons. Personal and occupational data were collected for all members of the cohorts. Causes of death were obtained for all cohort members dying from 1952 through 1957.

Major Findings: No findings to be reported as yet. Tabulation and some preliminary analysis of the data indicate that some differences exist in cancer mortality among occupational groups, although these differences are not dramatic. More detailed analysis of specific occupations may reveal greater differences.

Significance to NCI Program & Bio-Medical Research: This study is expected to yield cancer mortality data according to occupational and operational exposures for the largest industrial population ever studied on such a basis. It should indicate what types of exposure in this or similar industrial work may be expected to contribute to the incidence of cancer.

Proposed Course of Project: As soon as preliminary analyses are completed, decisions will be made as to the approach to be used in the final analysis. It is expected that the study can be essentially completed by August 1972.

Date Contract Initiated: 7-1-68

Current Annual Level: $18,000

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
FISCAL YEAR 1972

Office of the Chief
(With Colon Cancer Segment, Carcinogenesis)

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY (NIH-71-2427)

Title: Fecal Flora Studies

Contractor's Project Directors: Dr. W.E.C. Moore
Dr. Lillian V. Haldeman

Project Officer (NCI): Dr. Sidney J. Silverman

Objectives: To compare species of bacteria present in the feces of Japanese in Hawaii who have precancerous lesions with the flora of those without such tumors and with Japanese in Japan. Determine the effects of age, sex, and diet on the flora. Determine neutral and acid steroids in the feces of individuals under study.

Major Findings: In these beginning months of the project preliminary studies were performed to determine the effect of freezing fecal specimens under various conditions that could be used for shipping and storage. Since the majority of fecal microorganisms are strict anaerobes, the air in the collection bags was replaced with oxygen-free CO_2. Samples were stored for two days and for three weeks under liquid N_2 or on dry ice either suspended in glycerol-beef extract or in the solid state. When compared with storage under CO_2 at ambient temperature, no advantage was observed for frozen samples maintained for two days. Less change possibly occurred in the relative proportion of the kinds of organisms present at ambient temperature after two days. For longer periods of storage freezing was, of course, necessary. The results also indicated that the microorganisms may not be distributed homogeneously throughout the specimen, but rather that pockets enriched with a single species may occur.

Comparison of the fecal flora of two individuals (clinically normal North Americans) on a similar but not identical diet showed that each was characterized by a predominance of one species. Thus, specimens from "x" contained 31% Bacteroides and 10% Bifidobacteria (495 isolates studied), while, of those, from subject "o", 30% were Bacteroides and 39% were Bifidobacteria (266 isolates). A new species, named Ruminococcus bromii was identified in the samples from both persons.

On the basis of this limited study it was decided to initiate the bacteriological study of specimens from cohort subjects by

culturing fresh samples in Hawaii and returning the cultures to VPI for identification of the organisms. Undiluted specimens frozen in liquid N_2 are shipped also for steroid analysis and for further bacteriological study if necessary. The Japanese subjects are members of a cohort that has been under study by the National Heart and Lung Institute for a number of years.

Significance to Biomedical Research and Program of the Institute: These studies will indicate the inter-relationship between diet, microbial flora, and the induction of large bowel tumors. This is the first program that will have sufficient demographic data, clinical information, and laboratory findings to indicate the environmental factors leading to cancer of the colon. The preliminary studies, performed to obtain baseline data and to determine the procedures to be used, are probably the most complete study of the bacterial flora of the human intestinal tract made to date.

Proposed Course: The examination of specimens from cohort members will continue throughout the next fiscal year. Since members of the cohort are examined in a random fashion (according to a sequence established when the Hawaiian Heart Study originated), a continuing study will be required to obtain individuals with precancerous conditions and to obtain a sufficient number to relate diet and other factors to these conditions.

Date Contract Initiated: June 30, 1971

Current Annual Level: $151,707

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1972

ADP Management Section

MARYLAND NATIONAL OPTIMATION SERVICE, INC. (NIH-71-2050)

Title: Data Conversion for the Third National Cancer Survey

Contractor's Project Director: Daniel Wallingsford

Project Officer (NCI): Theodore Weiss

Objectives: Convert the data on approximately five hundred thousand Third National Cancer Survey documents to magnetic tape.

Major Findings: Data is being converted by the optical character recognition method (OCR). The contractor is using a system of procedures and computer programs specifically designed for him by the Institute. The system includes extensive security control over documents and information, a pre-edit of completed Survey documents, and manual and computer edit checks of the data transcription process.

Significance to Biomedical Research and the Program of the Institute: See Third National Cancer Survey Project Report, Special Cancer Survey Section, Biometry Branch, NCI.

Proposed Course: Contract is expected to be renewed through completion of the Survey (approximately September 30, 1973).

Date Contract Initiated: October 19, 1970

Current Contract Level: $169,700

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1972

ADP Management Section

MAUCHLY AND COMPANY, INC. (NIH-70-2062)

Title: Maintenance of Third National Cancer Survey Data Processing System

Contractor's Project Director: Thomas Marshall

Project Officer (NCI): Theodore Weiss

Objectives: Complete projects underway and assist in the turnover of system development, maintenance, and report preparation to the new data processing contractor, the Wolf Research and Development Corporation.

Major Findings: During this extension period, several computer programs were written to generate reports required by the Special Cancer Survey Section in its 1969 case completion program prior to the production of the Third National Cancer Survey, Preliminary Report of 1969 Incidence. In addition, the contractor completed those aspects of the computer system necessary to generate 133 separate reports, in camera-ready form, for the Preliminary Report of 1969 Incidence.

Significance to Biomedical Research and the Program of the Institute: See Third National Cancer Survey Project Report, Special Cancer Survey Section, Biometry Branch, NCI.

Proposed Course: The contract was terminated on August 27, 1971.

Date Contract Initiated: 12-22-69

Current Contract Level: $204,175

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1972

ADP Management Section

WOLF RESEARCH AND DEVELOPMENT CORP. (NIH-71-2270)

Title: Data Processing Support for Biomedical Research

Contractor's Project Director: William Lake, Ph.D.

Project Officer (NCI): Theodore Weiss

Objectives: This contract provides systems design and computer programming support to ten epidemiological research projects.

Major Findings:

The Epidemiology Branch Project - Computer programming and operational support was provided to the Epidemiology Branch's research activities. A special-purpose data processing system was modified and expanded to generate a series of tables from the Connecticut Cancer Registry file for a study of the Incidence of Second Cancers. A computer program was written in support of the U.S. Cancer Mortality Study to convert U.S. Vital Statistics Mortality data files to standard codes and fixed record formats and produce reports from this data base for special studies.

The Norwegian Migrant Study Project - Three computer files and three computer programs, received from a Norwegian Cancer Registry, were made compatible with DCRT conventions, and documented to form the data base and file processing system for the Norwegian Migrant Study. The contents of these files were verified and two new computer programs were developed to generate statistical tables.

The Study of Cancer Among Railroad Workers Project - A computer program was written to extract a 554,000 record subset of deceased employees from a 1.7 million record data base containing information on retired railroad workers. A table of frequencies by ICD code and occupational group was produced and a generalized data retrieval system was installed to facilitate the production of additional reports.

The Japanese Migrant Studies Project (JMS) - Computer programming support was provided to operate, maintain, and modify the JMS data processing system. Major modifications were made to the JMS system to conform to an operating system change required by DCRT and to accommodate additional information for two new studies, the Lung Cancer Study, and the Colon-Rectum Study. Several computer programs were written to generate special reports and reformat a subset of the data file so that investigators could interrogate and analyze the data from remote entry terminals.

The End Results Group Project - Computer programs were designed and implemented to produce special purpose reports for monitoring data submitted by the ERG registries for the End Results in Cancer Report #4. Several computer programs were developed, to generate a series of tabulations for the End Results in Cancer Report #4. A file independent computer system for the ERG which will calculate frequencies and age-specific and age-adjusted incidence rates utilizing user supplied parameters is currently being designed, and will be implemented during this fiscal year. Variables include base year, age, sex, race, year of diagnosis, and site grouping.

The Clinical Trials Project - Computer programming and statistical support was provided to the Clinical Trials research program. This involved the operation, maintenance, and modification of existing systems, the design and implementation of new programs and systems, and the statistical analysis of data.

The Third National Cancer Survey Project - Technical ADP support was provided to operate and maintain the ADP aspects of the Third National Cancer Survey data processing system and produce computer-generated reports for analysis and publication of Survey data. This included the technical direction of all activities involved in the conversion of data from some 340,000 source documents to magnetic tape, the operation and maintenance of the Third National Cancer Survey data processing system, and the design and implementation of new computer programs and systems to meet specific operational and analytical needs as they arose.

During this fiscal year Wolf:

- modified the 1969 Incidence Report system to produce variations of the basic reports for each Survey area and for different site and age groups;
- wrote new programs to produce special reports for each hospital participating in the Survey;
- developed, implemented, and operated a new computer system for processing data collected in the Special Skin Cancer Survey;
- modified the Third National Cancer Survey data processing system to process Hospital and Clinic Medical and Financial Supplement forms which contain medical treatment and cost detail for a 10% sample of the Survey's cases;

- monitored the operation of the Third National Cancer Survey data processing system which routinely processes approximately 5,000 new documents per week against a file presently containing over 700,000 records;

- recoded and tested changes to approximately 30 computer programs in the system to conform to the American National Standards Institute (ANSI) change in the COBOL programming language implemented by the Division of Computer Research and Technology;

- modified the system to accommodate version twenty of the IBM 360 and IBM 370 Operating System required by IBM and implemented by the Division of Computer Research and Technology;

- reprogrammed portions of the computer system to meet the Division of Computer Research and Technology requirement that all users establish and maintain their own computer program libraries.

The Japan-Hawaii Cancer Study Project - Technical ADP support was provided to design and implement a data processing system at the Kuakini Clinic and the University of Hawaii Computer Center. A prepackaged data processing system, National Information and Processing System (NIPS), was obtained from the Department of Defense, and converted to our specifications to reduce the amount of programming time required to develop and maintain a data processing system for this study. A NIPS data base was constructed of Honolulu Heart Study data taken from approximately fifteen separate Heart Study files. Several computer programs were written to generate tabulations and reports for use in the day-to-day operation of the Study.

Significance to Biomedical Research and the Program of the Institute: See Project Reports, Biometry Branch, NCI.

Proposed Course: The support represented by this contract is expected to continue through June 30, 1973.

Date Contract Initiated: 5-03-71

Current Contract Level: $444,000

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1971

Demography Section

YALE UNIVERSITY (70-2287)

Title: Analysis of Veterans Administration Urological Research Group Data

Contractor's Project Director: Dr. Colin White

Project Officer (NCI): Dr. David P. Byar

Objectives: Extension of methodology for an exponential survival model which incorporates concomitant information and application of this model to data from the Veterans Administration Cooperative Urological Research Group studies of treatment for cancer of the prostate.

Major Findings: During the past year and a half extensive work has been done extending work originally begun by us on the use of a multivariate exponential survival model. Specifically, we have studied a step-wise procedure for eliminating variables from the analysis in order of their importance in predicting survival based on the likelihood function. A goodness of fit test has been devised for checking the agreement of the model with the observed data, and the model has been tested by means of half-sample pseudoreplication. These results are currently being prepared for publication. We have also investigated the effects of including first order interactions in the model, and have found that with these data they do not add appreciably to the information. We have compared estimates of the variance of the parameters of the model based on the asymptotic variances with those obtained in half-sample pseudoreplication and found that when estimating three such variances on samples of only 100 observations, the two estimates were in good agreement.

Proposed Course: It is expected that this work will continue for a third contract year starting in July 1972, and that it will be completed in that year.

Date Contract Initiated: 6-1-70

Current Contract Level: $45,000

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1972

END RESULTS SECTION

CALIFORNIA DEPARTMENT OF PUBLIC HEALTH (69-5)
UNIVERSITY OF CALIFORNIA (67-62)
CHARITY HOSPITAL OF LOUISIANA (64-83)
UNIVERSITY OF CHICAGO (65-5)
CONNECTICUT STATE DEPARTMENT OF PUBLIC HEALTH (63-1148)
INDIANA UNIVERSITY HOSPITAL (70-2000)
STATE UNIVERSITY OF IOWA (64-15)
MASSACHUSETTS HEALTH RESEARCH INSTITUTE (72-2077)
UNIVERSITY OF MICHIGAN (66-24)
UNIVERSITY OF VIRGINIA (67-13)
NEW YORK MEDICAL COLLEGE (71-2424)

Title: End Results Evaluation

Project Officer (NCI): Dr. Sidney J. Cutler

Objectives: To obtain and analyze volume data for all cancer diagnoses, with analysis of specific types of cancer in patients with selected characteristics, in order to study:

1. the changes over time in the use of different methods of therapy and diagnostic criteria

2. the relative effectiveness of the various treatment modalities as judged by patient survival, and any changes over time in survival.

To acquire and analyze supplementary data designed to explain further any specific finding from the relatively crude mass data.

Methods Employed: The National Cancer Institute sponsors a program in which more than 100 hospitals contribute data on the end-results of cancer treatment. The Biometry Branch has professional and technical responsibility for supervising these contracts. Information on individual patients is submitted to the Biometry Branch, according to a prescribed schedule, and in accordance with a uniform punchcard code. This program makes available to the National Cancer Institute information on a very large series of patients (e.g., 644,000 patients were first treated in participating hospitals during this period 1935-1969) in a form which permits maximum flexibility in handling and analyzing the data, and also provides an extensive resource for special studies on selected series of cases. Partial support for three centralized registries (California, Connecticut, and Massachusetts) and for six registries in university hospitals is provided through the contract mechanism to make it possible for the participating registries to meet specified criteria regarding completeness, currency, and accuracy of information.

CONTRACT NARRATIVE - End Results Section (continued)

To obtain supplementary data, patients with pertinent characteristics are identified in the accumulated files. The medical records for these patients are reabstracted in detail using specially designed protocols. The records of similar patients may be abstracted on a prospective basis.

Major Findings: A firm answer to the question: "How many patients are being cured?" is unavailable because cancer can recur many years after treatment. The therapeutic success depends on diagnoses of cancer prior to spread beyond the site of origin. The most recent submission of data, as published in End Results in Cancer, Report No. 4, indicates that there are only a few sites for which there has been an increase in the percent of cancer diagnosed as localized since the 1950's:

Site	Percent Localized 1950-59	1960-64	1965-69
Female Breast	42	46	47
Prostate	52	60	63
Bladder	70	78	79
Melanoma of the skin	62	69	73
Brain	76	85	86

Survival data indicate continuing improvement for all these sites with the exception of breast cancer for which the 3-year relative survival rate has leveled off at slightly over 70 percent. Evidence of improvement in the management of cancer patients is indicated by improved survival observed for a larger list of sites despite the fact that for these other sites there is little evidence for "earlier" diagnosis:

3-year Relative Survival Rate (percent) for All Stages

Site	1950-59	1960-64	1965-69
Larynx	60	61	67
Prostate	59	64	66
Bladder	58	60	62
Melanoma of the Skin	63	69	74
Brain	28	33	37
Thyroid	81	85	86
Hodgkin's Disease	44	53	61
Multiple Myeloma	13	19	27
Chronic Lymphocytic Leukemia	49	50	53

It is encouraging that for a few sites around 90 percent of surgically treated patients without evidence of spread survive more than 5 years; e.g. 95 percent for uterine cervix and 97 percent for thyroid gland.

Significance to NCI Program and Bio-Medical Research: The End Results Program provides the most comprehensive information available on major trends in cancer treatment and associated results, and provides a base for a variety of special studies.

CONTRACT NARRATIVE - End Results Section (continued)

Proposed Course of Project: Continued attention will be given to reducing the time lag in reporting incidence data and in reporting survival data for disease entities for which therapeutic practice is changing rapidly, e.g., leukemia and Hodgkin's disease. Greater emphasis will be placed on developing additional population-based registries to provide a resource for a wide variety of epidemiologic and clinical studies.

	Date Initiated	Current Annual Level
CALIFORNIA DEPARTMENT OF PUBLIC HEALTH (69-5)	7-1-62	$328,563*
UNIVERSITY OF CALIFORNIA (67-62)	11-1-66	39,494
CHARITY HOSPITAL OF LOUISIANA (64-83)	10-1-63	47,476
UNIVERSITY OF CHICAGO (65-5)	7-1-64	37,993
CONNECTICUT STATE DEPARTMENT OF PUBLIC HEALTH (63-1148)	6-1-63	266,135
INDIANA UNIVERSITY HOSPITAL (70-2000)	7-1-63	54,516
STATE UNIVERSITY OF IOWA (64-15)	7-1-63	32,800
MASSACHUSETTS HEALTH RESEARCH INSTITUTE (72-2077)	1-1-72	97,041
UNIVERSITY OF MICHIGAN (66-24)	9-1-65	34,000
UNIVERSITY OF VIRGINIA (67-13)	7-1-66	39,619
NEW YORK MEDICAL COLLEGE (71-2424)	6-24-71	33,000*

*(FY 72 estimate)

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1972

Special Cancer Survey Section

UNIVERSITY OF ALABAMA MEDICAL CENTER (Birmingham) (69-49)
CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH (Berkeley) (69-51)
COLORADO DEPARTMENT OF PUBLIC HEALTH (Denver) (69-50)
COMMONWEALTH OF PUERTO RICO, DEPARTMENT OF PUBLIC HEALTH (San Juan) (69-72)
IOWA, UNIVERSITY OF (Iowa City) (69-42)
MEDICAL ASSOCIATION OF GEORGIA (Atlanta) (69-47)
MICHIGAN CANCER FOUNDATION (Detroit) (69-41)
UNIVERSITY OF MINNESOTA, SCHOOL OF PUBLIC HEALTH (Minneapolis) (69-45)
UNIVERSITY OF PITTSBURGH (69-43)
UNIVERSITY OF TEXAS, SOUTHWESTERN MEDICAL (69-40)
MAYO FOUNDATION (Minnesota) (70-2057)

Title: Third National Cancer Survey

Contractor's Project Director: See table on following page

Project Officer (NCI): Sidney J. Cutler, Sc.D.

Objectives: Procurement and initial processing of data for the Third National Cancer Survey

Major Findings: A preliminary report presenting data on 1969 incidence was presented to the National Cancer Advisory Council in October, 1971. An abridged version of this report is available for general distribution.

Significance to Biomedical Research and the Program of the Institute: See Individual Project Report NCI-4268

Proposed Course: All of the routine data collection activities in the survey field offices are running smoothly except for Colorado. In this survey office the state Civil Service regulation has impeded the hiring of staff. In addition, there was a change in supervisor, and the present supervisor had to be oriented as to the survey operations. Close attention is being given to this field office, and we are providing all the support we can.

The case finding effort for the 1969 cases has provided the field offices with experience in this facet of data collection. In addition, it highlighted weak spots, and all field offices have instituted corrective measures. This will make case finding for 1970 and 1971 data more efficient. The 1969 preliminary report is being widely distributed to all cooperating agencies. This has had the effect of renewing interest in the survey, and the agencies should be more cooperative.

CONTRACT NARRATIVE - Special Cancer Survey Section (continued)

The major effort for this fiscal year will be completion of the medical and financial reports on patients in the sample diagnosed in 1969. Data from these abstracts will be complementary to the 1969 preliminary report. A major problem has been getting the information on to the master data file. The programming of this operation, which included a large number of edits, was complicated and time-consuming. This was accomplished in late March, and the field offices were given computer printouts which notified them of where they were in the operation and what errors were present in the documents on file. This completed the programming for all of the documents, with the exception of the patient interview.

The medical and financial data will include information on all hospitalizations occurring within a two-year period of the date of diagnosis. They will include information on the type of therapy given the patient, including details as to the type of surgery and the extent of the disease at the time of diagnosis. The financial data will include all of the charges and how they were paid (personal funds, Medicare, insurance, or written off by the hospital).

In four of the field offices--Dallas, Iowa, San Francisco, and Minneapolis--a special skin cancer survey was started. In this survey, information on all patients with skin cancers diagnosed during the period September 1, 1971, through February 29, 1972, was abstracted. Special effort was made to obtain cooperation from physicians treating skin cancers, since a good portion of this population is not treated in a hospital, nor is the lesion histologically diagnosed. The forms used are different from the full survey, but contain detail as to the location of the lesion and the histologic type.

This skin cancer survey is independent of the full survey, and there is no correlation of the records or data even if a patient is reported in both surveys. It is hoped that data collection can be accomplished by the end of the summer and a report issued early in 1973.

A number of workshops were held during the year. The first, in November of 1971, was for the purpose of bringing the supervisors up-to-date and to discuss what has to be done in the near future. Emphasis was put on the priority to complete medical and financial abstracts for the 1969 cases. The second workshop, held in January of 1972, was for the purpose of training the abstractors how to code extent of disease. As a follow-up to this workshop, visits were made to each survey field office to review the completion of this part of the abstract.

Time tables are being developed for phasing out the survey. Within the next fiscal year, most of the abstracting from hospital records should be completed. Death certificate abstracts for deaths occurring in 1972 should be processed and follow-backs completed by mid-1973.

CONTRACT NARRATIVE - Special Cancer Survey Section (continued)

All hospitalizations of the sample patient that require medical and financial abstracts should be identified, and the abstracting should be nearly complete by September, 1973. The interview process should be started, and arrangements should be made for interviews to be completed before the end of the calendar year 1973.

As time is available to the survey central office staff, special analysis of the 1969 data, available on a special analysis file, will be done.

Date Contract Initiated: See table on following page

Current Contract Level: See table on following page

Contractor	Contractor's Project Director	Date Contract Initiated	Current Contract Period	Level
University of Alabama	Dr. Peter Peacock	10/ 1/68	9/ 1/71- 8/31/72	$ 67,857
California Dept. of Public Health	Mr. George Linden	10/ 1/68	9/ 1/71- 8/31/72	246,500
Colorado Dept. of Public Health	Dr. C. S. Mollahan	10/ 1/68	9/ 1/71- 8/31/72	118,000
Puerto Rico Dept. of Public Health	Dr. I. Martinez	12/ 9/68	12/ 9/71-12/ 8/72	9,537
University of Iowa	Dr. Edward Mason	10/ 1/68	9/ 1/71- 8/31/72	138,400
Medical Association of Georgia	Dr. James Cooney	10/ 1/68	9/ 1/71- 8/31/72	65,800
Michigan Cancer Foundation	Dr. Michael Brennan	10/ 1/68	9/ 1/71- 8/31/72	217,600
University of Minnesota	Dr. Leonard Schuman	10/ 1/68	9/ 1/71- 8/31/72	155,176
University of Pittsburgh	Dr. Carol Redmond	10/ 9/68	9/ 1/71- 8/31/72	128,110
University of Texas	Dr. Eugene Frenkel	10/ 1/68	9/ 1/71- 8/31/72	184,604
Mayo Foundation	Dr. Leonard Kurland	12/22/69	12/22/71-12/31/72	24,960

C. SUMMARY REPORT
EPIDEMIOLOGY BRANCH
JULY 1, 1971 through JUNE 30, 1972

The objective of the Epidemiology Branch is to test ideas concerning the origins of cancer through epidemiologic studies based on medical knowledge and experience and on an awareness of resources best available at the national or international level. An idea is developed from existing knowledge, which may come from the laboratory, the clinic, or other epidemiologic studies. Emphasis is placed on epidemiologic research based on important new information from laboratory investigations at the National Cancer Institute and elsewhere.

Staff Changes: Fred H. Burbank, M.D. and R. David Jensen, M.D. completed their two years of service as Staff Associates, and were replaced by Edward T. Creagan, M.D. and Gordon W. Grundy, M.D. Thomas J. Mason joined the staff as Statistician upon completion of his graduate work at the University of Georgia. Howard M. Hayes, DVM, transferred to the Branch from the FDA, and is now engaged in studies of veterinary cancer epidemiology.

Outside Training: Frederick P. Li, M.D. completed his training as a Fellow in Oncology at the New England Center Hospital in Boston.

Research Program:

Childhood Cancer: In 1962 the Branch began to examine the relationship between cancer and congenital defects. This epidemiologic approach, little used previously, seeks an excessive coexistence of the two diseases in persons, families or communities. When diseases are found to occur together more often than can be expected by chance, each can be studied in the light of what is known of the other for clues to etiology. In the past year several interesting associations between cancer and congenital defects were identified.

A large series of children with retinoblastoma showed an increased frequency of mental retardation, often associated with a complex of malformations suggesting deletion of a D-chromosome; this cytogenetic defect has been described in a few recent case-reports of retinoblastoma. Children with the eye neoplasm also had an apparent excess of double primary tumors, especially osteogenic sarcoma, which was not attributable to radiation. The study further revealed that nationally, from 1960-1967, an estimated 81% of children with retinoblastoma were cured. Among blacks, however, mortality was 2-1/2 times greater than among whites. This is the only tumor of childhood in which such a disparity occurs. Whether or not delay in diagnosis or treatment is responsible is as yet unknown.

A study of national mortality from testicular cancers in childhood and review of 70 medical records of children with the neoplasm revealed peak occurrences in infancy and puberty among whites, with no evidence of such peaks among black children. Fifteen of the 70 children in the hospital series had

inguinal hernias, undescended testes or other genitourinary defects, a frequency which appeared to be excessive. Other childhood cancers now being comprehensively studied include tumors of the brain and lymphoma.

Under the auspices of the International Union Against Cancer (UICC) we are making an international comparison of the occurrence of childhood cancer according to cell type. The purpose is to seek clues to etiology by identifying unusually high or low frequencies in various geographic areas. One tumor already known to exhibit a localized high frequency is Burkitt's (African) lymphoma.

Oncologists throughout the world have been most generous in contributing case abstracts to us for series of children's cancers in their locales. Many thousands of records have been abstracted in the U.S., Canada, Africa, Europe and Asia, with representation from Latin America somewhat less. Already it is clear that some important differences in cancer frequencies do exist. Interest in the study and in the etiology of childhood cancer was greatly stimulated by a description of the findings to date at the annual meeting of the International Society for Pediatric Oncology in Mainz, Germany in October 1971.

In the UICC study, upon receipt of data from a contributor, we organize the material in a standard format and compare the results with those from a large population-based registry with excellent pathological review of tumor tissue. Tabulations of the results are sent to the contributor along with comments as to how his data differ from those elsewhere. The comments may lead him to publish his observations or to investigate further any exceptional features noted.

Evaluation of the prenatal origins of certain cancers in man has revealed 8 lines of evidence for such an effect. Influences acting before the birth of a child include a drug (stilbestrol), ionizing radiation and inheritance. In addition, tumors may arise in the child from transplantation from the mother or, in the case of leukemia, from an identical twin. Other evidence of intrauterine origins of cancer include early age peaks in occurrence, co-existence with congenital malformations and the appearance of the tumor full grown at birth. These observations, many developed by the Branch, were presented at an international meeting on transplacental chemical carcinogenesis, sponsored by the International Agency for Research on Cancer in Hanover, Germany in October 1971.

Review of epidemiologic evidence for transmission of breast cancer by breast-feeding did not support the notion based on recent virologic studies that mothers with a family history of breast cancer should not nurse their daughters for fear of transmitting the neoplasm to them. The epidemiologic evidence to date runs counter to the hypothesis that such a risk is involved.

Several new projects were developed during the year. One of these we refer to as the "alert practitioner" program. It is an attempt to increase recognition of environmental agents that cause diseases of children through observations made at bedside. (History shows that it is not large-scale epidemiologic studies but alert practitioners who first recognize the links

between environmental agents and carcinogenesis or teratogenesis.) The program, developed by our Branch, will be supported under contract by NIEHS with the American Academy of Pediatrics (AAP). Feasibility studies will be conducted at 3 major pediatric centers. At each a faculty member will supervise 4 medical students as they collect information about environmental exposures from parents whose children have diseases of unknown etiology, including cancer, congenital malformations, and non-infectious dermatologic disorders. Every 6 months, the supervising faculty member will submit a summary report on his institution's observations to the Committee on Environmental Hazards of the AAP. The Committee will evaluate the findings and submit a report to the NIEHS. If an agent falls strongly under suspicion in a single case or a small series of cases at a participating hospital, the supervising faculty member will telephone a report to the Committee on Environmental Hazards for evaluation. If the finding is of special interest, the Committee will promptly inform NIEHS so appropriate technical resources may be made available to the original observer to clarify the relationship. It is expected that the program will serve four purposes:

1) Provide new information about environmental causes of childhood diseases,

2) Determine students with a special aptitude to think epidemiologically, so they may be singled out for additional training or career guidance,

3) Set examples of the relationships that can be found through this approach, and

4) Focus greater attention during medical-school training on etiology, which is, of course, a key to prevention.

Another project, being developed in conjunction with the Chemical Carcinogenesis Branch, NCI, concerns the health and well-being of long-term survivors of childhood cancer. With improvement in therapy, their numbers have been increasing substantially recently. A feasibility study has been developed to gather data on long-term survivors of Wilms' tumor at several large centers for children's cancer. The survivors will be evaluated for the occurrence of second primary tumors, a possible complication of chemotherapy, for other diseases, and, when the survivors reach the child-bearing age, the occurrence in their children of cancer or congenital malformations of the kidneys will be of special interest. Psychosocial effects on the child and his family will also be appraised. A similar study will be made of women with long-term survival after choriocarcinoma. In preparation for the study, a symposium is being planned to summarize current knowledge and to indicate areas in which research would be most profitable.

Adult Cancer, Not Occupational: Through the use of magnetic tapes provided by the National Center for Health Statistics, we have been analyzing data abstracted from the death certificates of the 4-1/2 million persons who died of cancer in the United States, 1950-67. We now have the capacity to study

cancer mortality by site, county of residence at the time of death, ethnic group, etc. Thus, we were able to seek confirmation of the claim that persons living in houses built on uranium-mill tailings were suffering an excessive mortality from cancer. Our study of cancer mortality by site and by county on the Western Slope of Colorado revealed no such excess attributable to radiation exposure.

Through the use of the magnetic tapes it was found that among American Indians, cancer mortality rates for males since 1950 had remained constant, as contrasted with substantial increases in rates for other ethnic groups in the United States. Especially infrequent were deaths from cancer of the lung, pancreas, stomach, colon and prostate. Among Indian females, cancer mortality showed 4 unusual features: 1) the average cancer mortality was substantially less than that for other U.S. ethnic groups, 2) mortality since 1950 had decreased 28% as compared with a decrease of 8% among whites, 3) mortality rates were substantially higher in Indians than whites for cancers of the liver and bile ducts, pancreas and uterine cervix, and 4) breast cancer mortality was about half of that experienced by other ethnic groups in the U.S.

Review of the carcinogenicity of drugs in man revealed that the evidence is sparse. In most instances, it is less than that for synthetic estrogens which cause adenocarcinoma of the vagina in the child, 14-22 years after the mother had been given heavy doses of the drug during pregnancy. To date 14 such cases have been documented in the literature. The evidence of chemical carcinogenesis in man is far greater following occupational exposures to chemicals than it is to drugs.

The Branch has now become known for its interest in family clustering of cancer either of the same cell type or of dissimilar cell types. Each week brings several reports by telephone or mail which our staff then investigate. The investigation involves creation of an accurate, detailed pedigree of the family and its illnesses, and the acquisition of specimens for analysis by laboratories at NIH. These specimens are subjected to tests often recently developed, to determine if healthy family members may show the same subclinical abnormalities as are found in their relatives who have cancer. If so, the subclinical abnormality may signify that that person although healthy at present, may be at increased risk of cancer. Knowing this makes possible more frequent examination in the hope that a tumor detected earlier than usual may be more amenable to therapy. For example, a family has been identified in which 7 and possibly 10 women have had ovarian cancer. Other women in the line of descent can now be examined frequently for early detection of the tumor, or, after they complete their families, prophylactic removal of their ovaries may be undertaken.

The Atomic Bomb Casualty Commission has made an immense contribution to cancer induction through its study of survivors of the atomic bombs in Hiroshima and Nagasaki. Recently it has been found that persons exposed to the bomb under 10 years of age are experiencing a much higher than usual cancer occurrence. To assure continuation and extension of these important studies, the Epidemiology Branch has provided funds through a contract with

the National Academy of Sciences which is administratively responsible for the ABCC. The contract seeks to increase Japanese scientific participation in the ABCC. Specifically, funds are provided to establish cancer tissue-registries in both cities. The absence of such a resource has been a handicap in the past. In Hiroshima, under the strong leadership of the President of the University, the possibility for success in this component of the contract is thought to be good. The contract provides funds also for several Fellowships in cancer epidemiology for young faculty members at Japanese universities. The Fellows will attend the Summer Session in Epidemiology for three weeks in Minneapolis, and after a short interval for orientation in Bethesda, will serve the remainder of the year at ABCC in projects that will enhance cancer epidemiology.

In collaboration with the Follow-up Agency of the National Academy of Sciences, which has access to data on the military-veteran population of the U.S. since World War I, data have been collected concerning the risk of cancer following selected viral and other conditions diagnosed during military service. During the year, two papers were completed: they showed no effect on cancer mortality following infectious mononucleosis diagnosed during World War II or in relation to yellow-fever vaccination, both of which involve potentially carcinogenic viruses.

Hycanthone is a drug that has been given to hundreds of thousands of people in Brazil as treatment for Schistosomiasis. Laboratory studies have shown the drug to be highly mutagenic, and suspicions are strong that it will also be carcinogenic. Because Atabrine is chemically related to Hycanthone, a quick evaluation was made of cancer mortality in relation to military service during World War II in areas where Atabrine was routinely used as prophylaxis against malaria. No unusual features of cancer mortality were found. However, NIEHS is planning a contract with the University of Maryland for study in Brazil of the effects of Hycanthone on the occurrence of mutations, congenital malformations and cancer. The Epidemiology Branch of NCI will participate in the development of the scientific design for the surveys to be conducted.

Under contract with the Johns Hopkins University, study is being made of cancer mortality among diabetics treated at the Joslin Clinic in Boston. Of particular interest will be the frequency of bladder cancer in recent years, since exposure to cyclamates has been heavier and longer among diabetics than in the general population. The study should be completed and a report written in the coming year.

Under contract with Harvard University, a test is being made of the hypothesis that the risk of breast cancer is affected by the relative levels of the individual estrogen fractions produced in the first decade after puberty.

A contract with Tulane University involves comprehensive histories and collection of serological specimens to test and to generate hypotheses concerning the origins of Hodgkin's disease.

Adult Cancer, Occupational: One staff member completed two years at the Occupational Health Field Station of the Public Health Service in Salt Lake City, where he supervised a reanalysis of data concerning lung cancer in uranium miners. A monograph on this subject appeared recently.

The Branch continued to encourage establishment of a National Death Index, which will, if adopted, be a tremendous stimulus to occupational studies in cancer. The leadership in seeking adoption of the Index has been taken by higher echelons of government, and though obstacles to implementation still remain, there is some hope that eventually the Index will be established. Without it, the opportunities for occupational studies are slim.

Veterinary Cancer: In 1961 the Epizoology Section was initiated in the expectation that research into neoplasia among domestic animals would provide new insight into the origins of human cancer. At that time, there was an absence of systematically collected data for epidemiologic research. The Section developed a system for making standard observations on a mass scale and to permit machine-processing and retrieval. The system was widely adopted both here and abroad. Virtually all U.S. veterinary medical colleges use it, and half of them send abstracts from each hospital or clinic admission record to our offices in Bethesda. Each contributing institution receives a monthly listing of its data; NCI has pooled data for its use.

Study of 8634 tumors among 202,277 animal patients seen through 1969 revealed several high risk categories (e.g., in dogs, large size was associated with high risk of total cancer). We have confirmed some findings by other investigators whose studies were based on smaller samples than ours, and have noted features which were not consistent with previous interpretations. For example, cancer eye in cattle was attributed to eye pigmentation and ultraviolet radiation; we have found a fourfold excess in females as compared with males, thus implicating for the first time an influence of sex on determination of this neoplasm.

Studies have been made particularly of four congenital defects primarily in dogs -- congenital hip dysplasia, patellar dislocation, eye defects and cardiac defects. The results verified previous reports of breed-specificity (e.g., patellar luxation in miniature breeds of dogs and hip dysplasia in large dogs), provided new hypotheses (e.g., one of the two types of congenital hip dysplasia in man corresponds to the single type seen in dogs), and defined new risk categories (e.g., the Samoyed breed of dog is at high risk of 1) atrial septal defect and 2) abnormalities of the pulmonic valve).

HONORS AND AWARDS

Robert W. Miller, M.D.

Committee Membership

National Council on Radiation Protection and Measurements
International Commission on Radiological Protection
  Committee on Radiation Effects
American Academy of Pediatrics
  Committee on Nutrition
  Committee on Environmental Hazards
National Academy of Sciences-National Research Council
  Committee on the Biological Effects of Ionizing Radiation
  Parent Committee, Subcommittee on Somatic Effects
  Subcommittee on Growth and Development
  Advisory Committee on the Atomic Bomb Casualty Commission
  Committee on Epidemiology and Veterans Follow-Up Studies

Consultant

Committee on Neoplastic Diseases - American Academy of Pediatrics
Atomic Bomb Casualty Commission, Hiroshima, Japan, November 28 - December 7, 1971.
International Agency for Research on Cancer, Workshop on Environmental Carcinogens. Geneva, Switzerland. December 12-18, 1971.
Children's Hospital of Philadelphia, Cancer Research Planning Group. February 4, 1972.
National Institute of Environmental Health Sciences: Contract to study carcinogenesis and teratogenesis in persons treated with Hycanthone for Schistosomiasis. Brazil. April 20-30, 1972.

Lectures

"Epidemiological studies of radiation injury," Annual Meeting of Health Physicists. New York, N.Y., July 13, 1971.
"Prenatal origins of childhood cancer: Epidemiologic evidence." International Symposium on Transplacental Carcinogenesis, sponsored by IARC. Hanover, Germany, October 5-7, 1971.
"International comparison of childhood cancer occurrence according to cell type," and "Etiology of childhood cancer." Annual Meeting, International Society of Pediatric Oncology, Mainz, Germany, October 8-9, 1971.
"Uses of an International Death Index." Annual Meeting, American Public Health Association, Minneapolis, Minnesota, October 14, 1971.
"Etiology of Wilms' tumor." Grand Rounds, Department of Pediatrics, Georgetown University. October 15, 1971.
"The environment and congenital malformations." Annual Meeting, American Academy of Pediatrics. October 18, 1971.
"Etiology of childhood cancer." Department of Epidemiology, Johns Hopkins University School of Public Health, Baltimore. November 12, 1971.

"Etiology of childhood cancer." Department of Pediatrics, Tokyo University. November 22, 1971.
"Etiology of cancer; epidemiological studies." NIH Seminar Series on Current Concepts in Oncology, Bethesda, Maryland. January 10, 1972.
International Symposium on Hodgkin's Disease. Stanford, California. (Discussant and Workshop Leader) March 20-24, 1972.
Chairman, Symposium on the Clinician and Cancer Etiology. American Society for Clinical Oncology, Boston. May 3, 1972.

Joseph F. Fraumeni, Jr., M.D.

Committee Membership

Biometry and Epidemiology Contract Review Committee, National Cancer Institute
Etiology Program Management Group, National Cancer Institute
Breast Cancer Working Group, Special Virus Cancer Program, National Cancer Institute
Scientific Advisory Committee, Cancer Control Bureau, New York State Department of Health
Cancer Research Training Committee, National Cancer Institute
Epidemiology Subcommittee, Breast Cancer Task Force, National Cancer Institute
NIH Committee on Transplantation Research
National Cancer Plan Conference, Project Areas Session (Objective 5, Approach 2)

Consultant

Advisory Committee on the Biological Effects of Ionizing Radiations, National Research Council - National Academy of Sciences

Lectures

"Epidemiology of leukemia." University of Pittsburgh, Graduate School of Public Health, Department of Epidemiology and Microbiology, Pittsburgh, Pennsylvania. September 9, 1971.
"Epidemiology of childhood cancer." Symposium on Cancer in Childhood, American Academy of Pediatrics, New York, New York. October 6, 1971.
"Who is at risk?" Panel on Epidemiology of Carcinoma of the Colon and Rectum, American Public Health Association, 99th Annual Meeting, Minneapolis, Minnesota. October 14, 1971.
"Peculiarities in the occurrence of childhood cancer," and "Viruses and childhood cancer." Fifth Annual Radiotherapy Symposium, Miami, Florida. December 10, 1971.
"Epidemiology and etiology of cancer." Department of Pharmacology, School of Medicine, George Washington University, Washington, D.C. January 24, 1972.
"Familial cancer." American Society of Clinical Oncology, Annual Meeting, Boston, Massachusetts. May 3, 1972.

William A. Priester, DVM

Committee Membership

Executive Secretary, Biometry and Epidemiology Contract Review Committee
Bioassay Advisory Committee, Etiology Carcinogenesis, NCI
Ad Hoc Committee for Bioassay Contract Review, Etiology, Carcinogenesis, NCI

Consultant

AID (Uruguay) "Animal Disease Reporting and Analysis"

Lectures

"The occurrence of tumors in domestic animals." Demography Seminar, Bethesda, Maryland. Summary from the NCI's Veterinary Medical Data Program. October 18, 1971.
"Occurrence of cancer in domestic animals." University of California School of Medicine, Davis, California. January 21, 1972.
"Using clinical data in research." University of California School of Medicine, Davis, California. January 21, 1972.
"The Veterinary Medical Data Program." University of Colorado, College of Veterinary Medicine, Fort Collins, Colorado. January 25, 1972.

John J. Mulvihill, M.D.

Committee Membership

National Academy of Sciences - National Research Council
Committee on the Biological Effects of Ionizing Radiation
Subcommittee on Growth and Development

Lectures

"Who is at risk of colorectal cancer?" (With Joseph F. Fraumeni, Jr., M.D.) Annual Meeting, American Public Health Association, Minneapolis. October 24, 1971.

Edward T. Creagan, M.D.

Lectures

"Cancer mortality among the American Indians: An 18-year survey." Demography Meeting, NCI, Bethesda, Maryland. February 29, 1972.
"Cancer mortality among the American Indians: An 18-year survey." American Association for Cancer Research, Boston. May 5, 1972.

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9. Fraumeni, J.F., Jr.: Viruses and childhood cancer. Proc. of Radiotherapy Symposium, Clinical Pediatric Oncology. Symposia Specialists. In press.

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Serial No. NCI-4325
1. Epidemiology Branch, OASDD, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Planning and Development in Cancer Epidemiology

Previous Serial Number: Same

Principal Investigator: Robert W. Miller, M.D.

Other Investigators: J. F. Fraumeni, Jr., M.D., W. A. Priester, DVM, and other professional staff on the Epidemiology Branch

Cooperating Units: None

Man Years:

Total	2.0
Professional	2.0
Other	0.0

Project Description

Objectives:

1. To originate new epidemiologic approaches to the study of cancer causation in man and animals.

2. To develop sources of data related to specific epidemiologic problems.

3. To stimulate epidemiologic research in other health agencies.

4. To develop on-the-job training for medical and statistical officers.

Methods Employed:

The Program is based on:

1. Leads from animal experimentation, laboratory research and clinical observations.

2. Prospective studies (in retrospect) which relate cancer occurrence to events recorded prior to the onset of cancer in medical examinations obtained in a standard fashion from large numbers of persons (examples -- clinical health-surveys, and military medical examinations).

3. Retrospective studies based on questionnaires obtained by personal interview or by mail, for a comparison of persons (examples -- clinical health-surveys, and military medical examinations).

4. "Laterospective" studies which concern the detection from clinic records of the excessive concurrence of cancer with pre-existent disease, such as congenital defects or autoimmune disorders.

As specific epidemiologic questions arise from laboratory or clinical observations, sources of field data are developed to answer these. Conversely, the Branch seeks by its surveys to raise questions which can be answered by laboratory or clinical studies. Parallel with this, to fill the tremendous deficiency in qualified personnel, the selection and training of young physicians, veterinarians, and statisticians must take place. The principles they learn from the guided planning, execution, and analysis of a program in cancer epidemiology are applicable to studies of the genesis of congenital malformations, heart disease, arthritis, mental aberrations, or the effects of ionizing radiation.

Significance to Bio-medical Research and the Program of the Institute:

If the example set by the Branch is a good one, interest will be stimulated among medical scientists in the use of epidemiologic methods for their research. In consequence, there may develop a further recognition of the usefulness of office and hospital records for survey studies. With this would go an appreciation for the need to adapt the standard medical records for epidemiologic research. These developments, in turn, could promote an interest in looking beyond the walls of hospitals and medical centers for opportunities in medical research.

Proposed Course of Project:

To develop opportunities in cancer epidemiology as they arise.

Serial No. NCI-4371
1. Epidemiology Branch, OASDD, Etiology
2. Epizoology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: National Cancer Institute Veterinary Medical Data Program

Previous Serial Number: NCI-4371

Principal Investigator: W. A. Priester, DVM

Other Investigators: H. M. Hayes, DVM

Cooperating Units: Schools and Colleges of Veterinary Medicine: Michigan State University, University of Missouri, University of Minnesota, Iowa State University, Ontario Veterinary College, Purdue University, University of Georgia, Kansas State University, University of Illinois, University of California, Ohio State University, University of Saskatchewan

Man Years:

Total	2.40
Professional	.40
Other	2.00

Project Description

Objectives:

To provide animal disease data as a research resource. To coordinate the program with related programs primarily in area of disease nomenclature, terminology and code. To develop ways and means of data utilization applicable to comparative medical studies.

Methods Employed:

Standard data processing methods. Details are described in related Project Reports and Contract Narratives of Epizoology Section.

Major Findings:

Not applicable as this is primarily a service activity. The National Cancer Institute Veterinary Medical Data Program is currently processing data from

10 veterinary colleges in this country and 2 in Canada. A modified program has been developed for diagnostic laboratories (pilot study Florida Diagnostic Laboratories). The program has been endorsed and implemented in Europe under the auspices of WHO. For the first time, veterinary medicine and the medical sciences have benefit of a national and international animal disease data system.

Significance to Bio-medical Research and the Program of the Institute:

The comparative (veterinary) medical data retrieval system instituted at the College of Veterinary Medicine, Michigan State University on a pilot basis (PH 43-64-85) was undertaken to design a system for collection, storage and retrieval of veterinary medical clinical data. The program is now fully operative and has been extended to 11 additional veterinary colleges. Establishment and maintenance of reliable sources of veterinary medical data provide a research resource, hitherto unavailable, that is applicable not only to cancer research but to research areas such as infectious diseases, congenital defects, and gerontological diseases. Continued NCI Epidemiology Branch animal studies research function depends upon continued availability of valid domestic animal disease data, particularly as regards neoplastic disease. The data bank, which included information on more than 500,000 patients through 1971, is often used as a reference source by scientists at NCI and elsewhere.

Proposed Course of Project:

Additional veterinary schools will be added to the program as interest and capability of the individual applicants are established. It is anticipated that this project will continue for a minimum of two years.

Date initiated: October 22, 1965

Current annual level: $21,000

Publications

Priester, W.A.: The management of clinical and laboratory data in veterinary medicine. Adv. in Vet. Sci. and Compar. Med. 16: (In press), 1972.

Serial No. NCI-4377
1. Epidemiology Branch, OASDD, Etiology
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Familial Aggregation of Malignancies

Previous Serial Number: Same

Principal Investigators: Joseph F. Fraumeni, Jr., M.D. and Robert W. Miller, M.D.

Other Investigators: John J. Mulvihill, M.D., Frederick P. Li, M.D., Gordon W. Grundy, M.D., and Edward T. Creagan, M.D.

Cooperating Units: None

Man Years:

Total	:	1.50
Professional	:	1.25
Other	:	.25

Project Description

Objectives:

To document the occurrence of patterns of family aggregation of neoplasms. To study such families by genetic and laboratory investigations, in an effort to elucidate the degree to which heredity and/or the common familial environment contribute to the etiology of neoplasms. To distribute tissue and blood specimens from such families to interested laboratory investigators, for etiologic study by cytogenetic, immunological, viral, and tissue culture methods. To identify genetic disorders predisposing to cancer.

Methods Employed:

Interview of cancer patients with respect to family occurrence of cancer and other disorders, as well as information on prior medical and environmental history; documentation of history by obtaining appropriate vital records and hospital charts; collection and distribution of biological specimens from such families; review of hospital records of series of patients with selected genetic diseases or neoplasms; survey of childhood cancer death certificates in the U.S. since 1960 to identify sib aggregation.

Major Findings:

A number of families have been studied with sites-specific cancers, such as familial lymphoma, breast cancer, ovarian cancer, chronic lymphocytic leukemia, chronic myelogenous leukemia, acute leukemia, cancer of the colon, and osteogenic sarcoma. Other familial occurrences have represented multiple cancer syndromes, including familial adenocarcinomas of multiple sites, and the familial syndrome of sarcoma, breast cancer and other neoplasms. Many of the tumors which occur together in familial syndromes also coexist excessively in studies of multiple primary cancer, suggesting that a familial cancer syndrome may represent a "scattering" over the family tree of tumors which share etiologic influences. The results of laboratory studies of specimens obtained from members of various cancer families are being assembled.

Significance to Bio-medical Research and the Program of the Institute:

Epidemiologic surveys and detailed studies of families at high risk of cancer may help to detect environmental and genetic influences in carcinogenesis. In addition, identification of these families has therapeutic implications, enabling surveillance and early diagnosis of neoplasms.

Proposed Course of Project:

The same approach will be continued. New laboratory methods and epidemiologic clues from other sources will be incorporated into the project protocol as necessary.

Serial No. NCI-4378
1. Epidemiology Branch, OASDD, Etiology
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: U.S. Cancer Mortality Survey

Previous Serial Number: NCI-4378

Principal Investigator: Thomas J. Mason

Other Investigators: Joseph F. Fraumeni, Jr., M.D., Frank W. McKay, Jr., and Edward T. Creagan, M.D.

Cooperating Units: National Center for Health Statistics

Man Years:

Total	:	3.25
Professional	:	2.75
Other	:	0.50

Project Description

Objectives:

To examine the cancer mortality experience in the United States, 1950-67 relative to cancer etiology for specific races, white, black, Indian, Chinese, Japanese, other, and also for individual counties within states.

Methods Employed:

Computer analysis of over 4.6 million death certificates by site, sex, race, state, and age. The investigation is ongoing, updated year by year, and expanding. To date, cancer sites have been analyzed by age, race, and geographic distribution. Base populations for whites, blacks and Indians are maintained for the entire time period. Racial comparisons are readily available. Standardized mortality ratios and the associated chi-square test are easily obtained. At the individual county level the capability of compressing the data into six 3-year intervals has been incorporated into the system. A measure of the standard error for each age-adjusted mortality rate is given routinely. Printer plots are available for age-adjusted mortality rates plus and minus their standard errors for individual tumor types and race-sex combinations as well as for selected groupings of tumor types.

Major Findings:

In an analysis of cancer mortality for the State of Colorado at county level, no relationship between cancer mortality and ionizing radiation due to uranium mill tailings used as construction fill since 1951 was detected. The fallacy of equating nonwhite with black has been pointed out, and has been utilized in an ongoing comparison of white and black cancer mortality patterns.

Significance to Bio-medical Research and the Program of the Institute:

This survey provides a continually expanding data set against which specific etiological theories about cancer can be tested. The capability of subdivision of the data set into specific racial and geographical subsets (specifically county level analysis) provides an opportunity to more critically test theories regarding the etiology of cancer than was possible at an earlier point in time.

Proposed Course of Project:

The provision of a working system of computer programs to facilitate the access, analysis, and display of results for any given subset of the mortality data available to the researcher is the goal of this project. A workable subset of tumor types has been agreed upon and will be utilized in subsequent analyses. An in-depth comparison between black and white cancer mortality is planned.

Publications

Mason, T.J., Fraumeni, J.F., Jr., and McKay, F.W.J., "Uranium Mill Tailings and Cancer Mortality in Colorado." (Submitted for publication)

Serial No. NCI-4379
1. Epidemiology Branch, OASDD, Etiology
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies of Congenital Defects in Domestic Animals

Previous Serial Number: NCI-4379

Principal Investigator: John J. Mulvihill, M.D.

Other Investigators: William A. Priester, DVM and Howard M. Hayes, DVM

Cooperating Units: See Project Report NCI-4371

Man Years:

Total	:	1.10
Professional	:	.70
Other	:	.40

Project Description

Objectives:

To study the characteristics of patients with congenital defects for clues as to possible relationships to cancer in the same clinic-hospital population. To relate these findings to human beings.

Methods Employed:

From case summaries submitted to the Veterinary Medical Data Program (NCI-4371), series of animals with congenital defects are systematically analysed in relation to the total number of animals seen in the same time period. A general survey of the findings has already been published for cases seen from March 1964 through January 1969. During this year, reports have been prepared on cardiovascular defects, hip dysplasia and patellar dislocation occurring in dogs during (for) the same period. Currently in progress are studies of progressive retinal atrophy, hernias, and congenital defects of the ocular, gastrointestinal, and urogenital systems, as well as an updated look at cardiovascular defects.

Major Findings:

Of animals seen at the participating institutions, about 5% have congenital

defects. In dogs the frequency of hip dysplasia was 17 per 1,000, of patellar dislocations, 8 per 1,000, and of heart defects, 4 per 1,000. In each series of animals, purebred dogs were at 2-3 times higher risk of having a defect than were crossbreds. Even among purebreds, certain breeds have even higher frequency of specific defects. For example, hip dysplasia was common in St. Bernards, German Shepherd Dogs, and Chesapeake Bay, Golden, and Labrador Retrievers (all large or giant breeds); whereas, patellar dislocation was more frequent in small breeds like Pomeranian, Pekingese, Chihuahua, Miniature/Toy Poodles, and Yorkshire and Boston Terriers. For each anatomic site of congenital heart disease, there seems to be a dog breed with a special predeliction: septal defects in Siberian Huskies, valvular defects in Chihuahuas and Golden Retrievers and patent ductus arteriosus in Miniature/Toy Poodles and Cocker Spaniels.

Significance to Bio-medical Research and the Program of the Institute:

Experiments on animals are essential to progress in medical research. Traditionally, a small number of species is used for laboratory experimentation. Currently popular laboratory animals are generally poor models for genetic disease and congenital malformations in man, and the rarity of these individual conditions in the human population limits clinical research. Our project attempts to detect conditions spontaneously occurring in domestic animals which are more nearly homologous to human afflictions. Taking heart malformations in dogs as an example, we have defined high frequencies of 1) patent ductus arteriosus in Miniature/Toy Poodles, and 2) persistence of the right aortic arch in German Shepherds. Since certain congenital malformations in man are associated with childhood malignancies, we hope to identify domestic animals which may serve as research models to explore the borderland between teratology and oncology.

Proposed Course of Project:

Data will continue to come from the Veterinary Medical Data Program and will be analyzed for breed-specific lesions.

Publications

Priester, W.A.: Sex, size, and breed as risk factors in canine patellar dislocation. J. Amer. Vet. Med. Ass. 160: 740-742, 1972.

Priester, W.A. and Mulvihill, J.J.: Canine hip dysplasia: Relative risk by sex, size, and breed, and comparative aspects. J. Amer. Vet. Med. Ass. 160: 735-739, 1972.

Mulvihill, J.J.: Comments on the epidemiology of congenital heart disease in dogs. In Heart: The Clinical Delineation of Birth Defects. Birth Defects: Original Art. Series, National Foundation, New York. (In press)

Mulvihill, J.J.: Congenital and genetic disease in domestic animals. Science (In press)

Serial No. NCI-4380
1. Epidemiology Branch, OASDD, Etiology
2. Epizoology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Study of the zoographic characteristics of domestic animals with tumors.

Previous Serial Number: NCI-4380

Principal Investigator: W. A. Priester, DVM

Other Investigators: None

Man Years:

Total	:	1.40
Professional	:	.40
Other	:	1.00

Project Description

Objectives:

To provide a reference of the distribution of spontaneous tumors in domestic animals. To search for unusual distribution of tumors, within a large, animal hospital population, which might suggest possible clues regarding the etiology of cancer.

Methods Employed:

Using patients with tumors reported to the Veterinary Medical Data Program as case series, and all patients seen during like periods as population references, the following will be studied:

Characteristics of patients: age, breed/species, sex
Characteristics of tumors: degree of malignancy, site, and histogenic type.

In addition, other factors may be studied as the analyses progress.

Major Findings:

8,634 tumors were reported during the period March 1964 through December 1969 among 202,277 patients seen. The proportion of patients with tumors was

highest among dogs and lowest among horses, when the four major species categories, bovine, equine, feline, and canine, were considered. A significantly increased tumor risk was noted in 1 bovine, 2 equine, and 14 canine breeds as compared to all breeds within each species category. Large breeds of dogs tended to be at higher risk for tumors than were small dogs. All 4 species showed an increasing risk for tumors with age; the equine species, however, showed much less age dependence than the other 3. Female cattle were at excess risk for malignant tumors.

Significance to Bio-medical Research and the Program of the Institute:

Animal studies done by the Branch have demonstrated several relationships of interest in cancer research -- bone sarcoma and size, canine lymphoma and certain breeds, canine mastocytoma and breed ancestry. This study is designed to detect high risk groups in a systematic fashion. The results may well define animal subgroups suitable for studies of cancer etiology that would be impractical in a human population.

Proposed Course of Study:

It is anticipated that the results from the current population under study, 1964-1969, can be used to generate hypotheses that will be tested in the 1970-1971 study population. It is expected the project will continue for a minimum of 3 years.

Date initiated: May 1970

Current annual level: $19,000

Publications

Priester, W.A. and Mantel, N.: Occurrence of tumors in domestic animals. Data from 12 United States and Canadian colleges of veterinary medicine. J. Nat. Cancer Inst. 47:1333-1344, 1971.

Serial No. NCI-4400
1. Epidemiology Branch, OASSD, Etiology
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Epidemiology Branch Field Station, Boston, Massachusetts.

Previous Serial Number: None

Principal Investigator: Frederick P. Li, M.D.

Other Investigators: None

Man Years:

Total	:	1.8
Professional	:	1.0
Other	:	0.8

Project Description

Objectives:

To study the epidemiology of human neoplasms and to develop etiologic hypotheses through close observation of individual patients at the bedside.

Methods Employed:

The Field Station personnel maintain close contact with several tumor therapy services in Boston through participation in conferences, teaching programs and clinical investigation. Patients under treatment at these centers are screened by means of chart reviews and by personal and questionnaire interviews. Clues to the origins of human cancers derived from these observations are studied using clinical, laboratory and epidemiologic methods.

Major Findings:

Familial aggregates of specific cancers, including breast cancer in males, medullary thyroid carcinoma and soft-tissue sarcoma, have been identified for further epidemiologic and laboratory investigation. Children with aplastic anemia were found to be at high risk of developing acute myelocytic leukemia. Risk factors for several other malignancies are being sought in retrospective chart reviews.

Significance to Bio-medical Research and the Programs at the Institute:

Research at the Field Station complements studies of cancer etiology by other clinical and laboratory investigators at the Institute. Epidemiologic studies help to examine the applicability of data in laboratory animal to human populations, and generate new etiologic hypotheses for testing by clinical and laboratory colleagues.

Proposed Course of Project:

The Field Station will continue with studies on the patterns of occurrence of human cancers. The emphasis will be on detection of clues to etiology through careful clinical study of the natural history of cancer in individual patients. Protocols are being developed to study newly diagnosed patients to collect data unavailable from retrospective reviews. Dr. Li, who is of Chinese ancestry and speaks Cantonese, is also initiating studies on cancer among the Chinese in the United States, with the goal of developing future international studies on the Chinese.

Serial No. NCI-4401

1. Epidemiology Branch, OASDD, Etiology
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Cancer Mortality Among the American Indians: An 18-Year Survey

Previous Serial Number: None

Principal Investigator: Edward T. Creagan, M.D.

Other Investigators: Joseph F. Fraumeni, Jr., M.D.

Man Years:

Total	.60
Population	.50
Other	.10

Project Description

Objectives:

To obtain a more precise understanding of the hereditary and environmental determinants of Indian cancer mortality. To identify unusual geographic, chronologic and familial aggregates of malignancy which might provide important clues to the origins of the neoplastic process.

Methods Employed:

Computer tapes provided by the National Center for Health Statistics to the National Cancer Institute contain information from death certificates of persons who died from cancer in the United States from 1950 through 1967. Abstracted from each death certificate were the cause of death, sex, race, age, and county and state of usual residence. Code numbers 140-205 of the International Classification of Diseases (ICD) represented the deaths from cancer. Deaths were coded according to the sixth revision of the ICD code during the years 1950-57, and to the seventh revision during 1958-67. To maintain comparability over the study period, all deaths from cancer in the latter interval were converted to the sixth revision code. However, the seventh revision has a special advantage of distinguishing between cancers of the liver and biliary tract, and was used in a separate analysis to evaluate mortality from cancers of these sites.

Bureau of the Census provides age-specific and total population for whites, blacks and nonwhites. The age distribution of the 1960 Indian population

was obtained from the Bureau of Indian Affairs; age-specific populations were calculated by applying this distribution to the total Indian population for the census years 1950, 1960 and 1970. Census figures for the total United States Indian population in five-year age groups were not available for these census years. Populations for the intercensal years were obtained by linear interpolation between census years for specific age groups. The 1960 population for each specific race was used to calculate the age-adjusted rates by the direct method.

Age-specific rates for the white and black populations were calculated for the entire 18-year period, 1950-67. These rates were then applied to the Indian population at risk for this period of time in order to calculate the standardized mortality ratio (SMR). We employed a standard chi-square test to evaluate the hypothesis that the Indian mortality experience was identical with that of the white or black population.

Geographic and time trends are presented. The 18-year pattern for selected tumor sites compares age-adjusted mortality for whites, nonwhites and Indians. Analysis of liver and gallbladder cancer only included 1958-67. An investigation of the residence at time of death provided a geographic profile of the Indian cancer mortality. The Bureau of Indian Affairs provided population data for those tribes in non-reservation states, especially in the east and south.

Major Findings:

Analysis of 5897 cancer deaths among American Indians between 1950 and 1967 revealed an age-adjusted mortality (per 100,000) of 64.7 as compared with 152.8 for whites and 124.8 for blacks.

The Indian male cancer mortality has remained constant since 1950, whereas the corresponding rates for whites and nonwhites have increased by 14% and 51%, respectively. Cancer of the gallbladder was the only tumor site for which the observed deaths (84) exceeded the expected (28) from white rates ($p < .01$); deaths from testicular, renal and gallbladder cancer were significantly higher than expected from the black rates ($p < .01$). From both white and black rates, mortality from cancer of the lung, pancreas, stomach, colon and prostate was especially low among Indian males.

Cancer mortality among Indian females was noteworthy in four ways: 1) Average annual age-adjusted mortality (per 100,000) of 70.1 contrasts with 141.3 for whites and 109.2 for nonwhites. 2) Cancer mortality has decreased 28% since 1950 vs. a decrease of only 9% among whites; rates in nonwhite females since 1950 have been constant. 3) Compared with white rates, mortality from gallbladder and bile duct, stomach, pancreatic, and cervical cancer exceeded the expected; the 57 deaths from kidney cancer were significantly higher than the 43 expected from black rates ($p < .01$). 4) Breast cancer mortality was only 45% of that expected for whites and 50% for nonwhites.

Significance to Bio-medical Research and the Program of the Institute:

The cancer experience of the American Indian provides opportunities for additional epidemiologic and clinical research. First, the Indian is a relatively well-defined ethnic group with a specific geographic distribution. Both features facilitate population-wide surveys. Also, a centralized agency, the Indian Health Service, not only delivers health care but accumulates data which may be of epidemiologic value. Second, recent studies demonstrate that chenodeoxycholic acid can effect the dissolution of cholesterol gallstones. Additional clinical trials of this agent would seem appropriate for the American Indian, a population in which biliary neoplasm is one of the leading causes of cancer mortality.

Proposed Course of Project:

A more refined analysis of Indian deaths from cancer will expand the present survey. First, the 1970 census specifically enumerates "American Indians" and will provide more accurate age-specific and age-adjusted mortality rates. Second, examination of the county of death for each tumor may detect unusual aggregates of cancer mortality among Indians.

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1972

Ecology Studies Section

HARVARD UNIVERSITY SCHOOL OF PUBLIC HEALTH (NIH-71-2179)

Title: Epidemiology and Etiology of Breast Cancer

Contractor's Project Director: Brian MacMahon, M.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D.

Objectives: To expedite and expand ongoing epidemiologic studies directed towards understanding the etiology of breast cancer and the identification of a preventive measure. At the present time, the studies are focusing on the specific hypothesis that estrogen profile during reproductive life is an important determinant of breast cancer risk.

Methods Employed: The human estrogen fraction studies involve the following: 1) an international study, in which estrogen-fraction determinations in urine involve comparison between women in 2 North American and 3 Asian populations; 2) a Hawaii "migrant" study, in which estrogen fraction determinations are made on migrant populations; 3) a degradation study to evaluate the stability of estrogen fractions in frozen urine; 4) an examination of additional Boston study groups (women of Chinese descent, and daughters of breast cancer patients) to analyze urinary estrogen and androgen profiles; and 5) identification of a potential prospective study group. Furthermore, an experimental study of rats is being conducted to clarify the carcinogenic and protective potential of estrogen fractions.

Major Findings: A preliminary analysis of the international survey was consistent with the hypothesis: The ratio of E3 to E1 and E2 was low in the areas of high breast cancer risk (Lancet 2:900, 1971).

Significance to NCI Program and Bio-medical Research: Epidemiologic characteristics of breast cancer are consistent with hormonal determinants, which can be best evaluated by joint epidemiologic-laboratory investigations of the type planned by this contract.

Proposed Course of Project: Preliminary evidence indicates that the estrogen-fraction hypothesis should continue to be evaluated. Investigations of other hormonal determinants, particularly androgen profiles, will be incorporated into the project. Under present plans, the contract will be shifted next year to the Epidemiology Subcommittee of the Breast Cancer Task Force.

Date Contract Initiated: June 23, 1971

Current Annual Level: $109,943

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1972

Ecology Studies Section

JOHNS HOPKINS UNIVERSITY (NIH-70-2134)

Title: Epidemiological Study of Cancer Mortality Among Diabetics

Contractor's Project Director: Irving I. Kessler, M.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D.

Objectives: To evaluate over time the cancer mortality experience of diabetics, whose per capita consumption of cyclamates since 1960 has greatly exceeded that for the general population.

Methods Employed: Utilizing a group of 21,447 diabetics registered at the Joslin Clinic in Boston over a 26-year period, the risk of deaths from all forms of cancer in the precyclamate and postcyclamate periods are compared with one another and with the rates prevailing in the general Massachusetts population.

Major Findings: Since 1950, the cancer mortality experience of diabetics has been similar to that occurring before 1960. Pancreatic cancer remains the only neoplasm that significantly exceeds the rates in the general population. No increases in bladder cancer have been identified.

Significance to NCI Program and Bio-medical Research: The Epidemiology Branch is concerned with studies relating environmental agents to cancer, and the surveillance of populations exposed to potential carcinogens. A widely used food additive, cyclamates, was found to cause bladder tumors in experimental animals; its carcinogenic potential in man is unknown, but may be clarified by a long-term survey of groups, such as diabetics, with heavier than normal exposure to cyclamates. This study also provides an opportunity to assess the risk of various types of cancer among diabetics.

Proposed Course of Project: It is expected that the follow-up survey and the mortality analysis will be completed within the next fiscal year.

Date Contract Initiated: March 1, 1970

Current Annual Level: $29,200

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1972

Ecology Studies Section

NATIONAL ACADEMY OF SCIENCES (PH43-64-44)

Title: Epidemiologic Studies in Etiology of Cancer in Veterans

Contractor's Project Director: Gilbert W. Beebe, Ph.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D. and Robert W. Miller, M.D.

Objectives: To develop and conduct a broad program of epidemiologic studies in cancer among veterans.

Methods Employed: The Epidemiology Branch of the National Cancer Institute and the Follow-up Agency of the National Academy of Sciences will develop an epidemiology program designed to make efficient use of the military-veteran population, utilizing medical, demographic, and environmental observations made at entry and during military service, and the subsequent medical history of veterans ascertained through facilities of the Veterans Administration and supplemented by mortality data. Four separate activities were carried out during the first year: 1) an epidemiologic study of Hodgkin's disease developing after two or more years of military service, comparing 750 cases and 750 controls with respect to variables ascertained from pre-service and inservice records; 2) an evaluation of familial factors in short life span and cancer by means of a twin registry of 16,000 pairs of veteran twins born in 1917-27; 3) preservation on magnetic tape of the Army diagnostic index to medical experience of the Korean War; and 4) preparation of reports on data developed from earlier studies of viral and other factors in etiology of cancer among Army veterans.

Major Findings: Reports have been prepared on the lack of relationship between cancer and histories of (1) infectious mononucleosis and (2) exposure to yellow fever vaccine (containing avian leukosis virus).

Significance to NCI Program and Bio-medical Research: The Epidemiology Branch of the National Cancer Institute is concerned with studies to identify and clarify etiologic factors in cancer, and these objectives can be more readily achieved by coordinating efforts with the outstanding resources and staff of the Follow-up Agency.

Proposed Course of Project: The specific projects noted above will be completed during the coming year; the Agency will maintain a continuing surveillance over opportunities presented by the military-veteran population for research in cancer etiology, and new projects will be generated over the next few years.

Date Contract Initiated: June 28, 1971

Current Annual Level: $92,200

Ecology Studies Section

TULANE UNIVERSITY SCHOOL OF MEDICINE (NIH-71-2423)

Title: Epidemiology of Lymphomas and Multiple Myeloma

Contractor's Project Director: Guy R. Newell, M.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D.

Objectives: To conduct a multi-hospital and prospective-incidence study of lymphomas and myeloma in Louisiana looking for clues to disease etiology.

Methods Employed: A questionnaire was developed for multi-hospital case control study of Hodgkin's disease to investigate relationships to various factors such as tonsillectomy and appendectomy, prior immune status, allergic conditions, smoking, and birth characteristics. For each individual, serum was drawn for E-B virus antibody determinations and HL-A histocompatibility antigens; pathology slides were collected for histological classification. In the incidence study all Louisiana residents with Hodgkin's disease were ascertained, to evaluate demographic variables, seasonal variation, clustering, and other factors.

Major Findings: No specific answers have yet resulted from the study. Interviews have been conducted on 221 individuals, including 87 cases of Hodgkin's disease. Sera have been collected on 218 cases. A study protocol has been designed for the prospective-incidence study.

Significance to NCI Program and Bio-medical Research: The Epidemiology Branch is concerned with studies relating environmental agents to cancer, including identification of etiologic influences in the lymphomas. There have been recent reports from Albany on the relationship of Hodgkin's disease to tonsillectomy, and a tendency for some cases of Hodgkin's disease to occur in an "extended epidemic." These observations indicate the importance of continued epidemiologic and etiologic studies of this cancer.

Proposed Course of Project: The project director and staff have demonstrated the feasibility of an epidemiologic study of Hodgkin's disease and other lymphomas in Louisiana. It is planned that the contract will continue for one more year, and then funding should switch to a grant mechanism.

Date Contract Initiated: June 29, 1971

Current Annual Level: $60,045

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1972

Ecology Studies Section

TULANE UNIVERSITY SCHOOL OF MEDICINE (NIH-71-2428)

Title: Study of Development of Cancer in a Cohort of Women Utilizing a Family Planning Facility

Contractor's Project Director: Guy R. Newell, M.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D.

Objectives: The project was a feasibility study to evaluate the risk of cancer, especially of reproductive sites, among women receiving oral contraceptives.

Methods Employed: The feasibility study attempted to determine whether it was possible to evaluate the cancer risk by follow-up of 25,000 women attending family planning clinics in New Orleans.

Major Findings: The feasibility study revealed that it was not possible to accomplish the objectives by utilizing the program at the family planning clinics. The retention rates for participating women were too low, a comparison group of women who never use oral contraceptives was too small, and the volume of patient data to be screened and recorded by hand was too great.

Significance to NCI Program and Bio-medical Research: The Epidemiology Branch is concerned with studies relating environmental agents to cancer, and the surveillance of populations exposed to potential carcinogens. It was hoped that the project could elucidate the risk of cancer in a population of women using contraceptive drugs, which have been suspected of carcinogenicity.

Proposed Course of Project: Since the feasibility study revealed that it was not possible to initiate the follow-up survey, it was decided to terminate the contract.

Date Contract Initiated: June 29, 1971

Date Contract Completed: June 29, 1972

Current Annual Level: $52,002

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1972

Ecology Studies Section

WESTERN RESERVE UNIVERSITY (PH 43-64-524)

Title: Mortality Experience of Children who were Inadvertently Inoculated with SV-40 very Early in Life

Contractor's Project Director: Eli Gold, M.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D.

Objectives: To determine whether the inoculation of human infants with polio vaccine containing SV-40 at birth is associated with an increased mortality due to malignant neoplasms.

Methods Employed: The mortality experience of 1,077 mainly Negro children, born in Cleveland, Ohio, between December 1959 and May 1962 and inoculated with SV-40 shortly after birth, will be followed through time. The location and name of each child are determined annually. Deaths are discovered through the use of a continuously up-dated census of Cleveland school children and from the vital records of the State of Ohio and other states to which the children have migrated. The names in the study group are compared against an alphabetic file of all childhood cancer deaths in the U.S. maintained in the Epidemiology Branch, NCI. The frequency of cancer deaths in the study group will be compared with the expected cancer mortality for children in the State of Ohio.

Major Findings: As yet there have been no cancers or other disorders in the study group attributed to SV-40. An interim report of the findings was published in Science 167: 59-60, 1970.

Significance to NCI Program and Bio-medical Research: Previous investigations by the Epidemiology Branch of school-age children who were inadvertently inoculated with SV-40 will be extended. The known importance of neonatal exposure in the causation of many viral neoplasms of animals suggests the possibility that the cancer mortality of children inoculated at birth might be different from that of children exposed at school age. The present study will extend our present knowledge in this area.

Proposed Course of Project: Since neoplasms with a latent period longer than 6 years could not be detected by the current follow-up, surveillance of the children will continue to be maintained.

Date Contract Initiated: March 9, 1964

Current Annual Level: $9,251

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1972

Special Cancer Studies Section

ALBANY MEDICAL COLLEGE OF UNION UNIVERSITY (NIH-71-2426)

Title: Study of Histopathologic Epidemiology of Childhood Cancer

Contractor's Project Director: Dr. J.N.P. Davies

Project Officer (NCI): Robert W. Miller, M.D.

Objectives: To carry out an international study of the incidence and frequencies of childhood cancer and to determine the causes of the striking variation in incidence from one country to another.

Methods Employed: Data on all childhood cancers is currently being collected from about 20 different countries. This statistical information will be analyzed by cell type and compared with data from other centers. A histologic review of the tumor types will be carried out when specific neoplasms may provide a basis for comparison, such as ovarian tumors and lymphomas which vary greatly from country to country. The principal investigator will request blocks or sections of certain tumors for this purpose.

Major Findings: Because this particular project is so new, no specific findings or results have been substantiated. Only the original hypothesis, that childhood cancers vary from one country to the next, not only in frequency, but also in cell type is now of prime interest. A prime example of this variation is the high frequency of Burkitt's lymphoma in Africa, or the almost non-existent risk of Ewing's tumor among U.S. Negroes.

Significance to NCI Program and Bio-medical Research: Marked variations in the frequency of cancer according to race and geographic origin provide opportunities to identify genetic and environmental factors in etiology. The Epidemiology Branch has been involved in epidemiologic studies of childhood cancer in the U.S. The detection and clarification of international variation in childhood cancer should increase the opportunities for generating etiologic leads. The proposed histopathologic studies should play a very integral part in illuminating these leads.

Proposed Course of Project: As data continues to be received from various cancer registries, hospitals and individual investigators from each country, tabulations and correlations will be made to elicit possible clues to origin and genetic relationships. Future analyses and further data solicitation will be made as deemed necessary to the total project.

Date Contract Initiated: June 24, 1971

Current Annual Level: $34,970

CONTRACT NARRATIVE
(Summary of Personal Service Contracts)

Animal Studies

VETERINARY MEDICAL DATA PROGRAM

Personal Service Contracts between the Epizootiology Section and Colleges of Veterinary Medicine at the University of Missouri, Michigan State University, Iowa State University, Ontario Veterinary College, and the University of Georgia, University of Illinois, Kansas State University, Ohio State University, University of Saskatchewan, Purdue University, University of Minnesota, and University of California.

Title: Purchase Veterinary Case Data.

Objectives: To add to volume of stored data, available to Epizootiology Section, in the Veterinary Medical Data Program. To further encourage adoption of standard nomenclature and coding of animal disease data. To make available to veterinary medicine, comparative medical research and the scientific community the developments of the Veterinary Medical Data Program.

Methods Employed: Purchase of completed veterinary medical case abstracts in terms of National Cancer Institute Veterinary Medical Data Program (as detailed in related Contract Narratives).

Major Findings: None, as this is primarily a service activity.

Significance to NCI Program and Bio-medical Research: Increase store of data in the Veterinary Medical Data Program. Of particular importance in terms of study of low prevalence diseases (cancer, etc.), geographical distribution and absolute numbers.

Proposed Course of Project: Minimum of two years.

Dates Initiated:

Michigan State University	- November 1965
Iowa State University	- October 1965
Ontario Veterinary College	- July 1966
University of Georgia	- January 1967
University of Missouri	- February 1967
University of Minnesota	- December 1967
University of Illinois	- January 1968
Kansas State University	- January 1968
Purdue University	- January 1968
Ohio State University	- January 1969
University of Saskatchewan	- January 1969
University of California	- July 1970

Current Annual Level: $30,000

ANNUAL REPORT

CARCINOGENESIS PROGRAM
ETIOLOGY AREA
NATIONAL CANCER INSTITUTE

July 1, 1971 through June 30, 1972

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I. *CARCINOGENESIS PROGRAM SUMMARY REPORT*

A. *INTRODUCTION*

The Carcinogenesis Program is responsible for planning, implementing, and managing the National Cancer Institute's coordinated research program on carcinogenesis by chemical and physical factors and on its prevention. In order to meet this responsibility, five coordinated program approaches have been established:

1. Identification and characterization of *population groups at risk* for different cancers;

2. Identification of carcinogenic activity of selected agents by *bioassays*;

3. Development and selection of *biological models* for bioassay, for the characterization of carcinogenesis processes, and for correlation with man;

4. Identification of *processes* required for carcinogenic action of selected agents as target points for corrective measures in man; and

5. Development, application, and evaluation of *corrective measures* for man and his environment.

The Carcinogenesis Program consists of an *intramural* component devoted to laboratory research, scientific documentation and coordination, and an *extramural* component devoted primarily to directed targeted research implemented through the contract mechanism. There is no programmatic distinction between the two except from an administrative standpoint. They are, in fact, designed to complement each other.

Historically, this Program developed from the Carcinogenesis Studies Branch established in March 1961 as part of the Field Studies Area, for intramural and collaborative investigations on carcinogenic factors, particularly as hazards in the human environment. With the reorganization of NCI and the establishment of the Office of the Scientific Director for Etiology in January 1966, the Carcinogenesis Program was organized under the Office of the Associate Scientific Director for Carcinogenesis (OASDC), with the task of "planning and administering a program of basic and applied research in carcinogenesis leading to the identification or definition of environmental carcinogens, and to the elucidation of carcinogenesis mechanisms." A Biology Branch and a Chemistry Branch were established to implement this program. A contract-supported collaborative program was developed since 1962 to respond to the need for extensive bioassay testing and screening of potential environmental chemical carcinogenic hazards, and for a variety of fundamental research programs on mechanisms of carcinogenesis.

The scope of the whole Carcinogenesis Program was reassessed in 1968. Following an extensive survey of the leading scientific centers in this field and a series of discussion panels convened by NCI at the request of the National Advisory Cancer Council, a Program Plan on Chemical Carcinogenesis and Prevention of Cancers was designed and approved: new directions were thus established for both intramural and collaborative activities.

At the end of FY 1968, the Carcinogenesis Program had a total personnel strength of 105 positions, all but a few totally engaged in laboratory work, often loosely related to the newly established program priorities. The contract program was at a funding level of about $5 million, with extremely limited coordination and management resources. The task of developing a highly coordinated targeted program, requiring full participation of scientific staff, development of new major program areas and establishment of managerial and technical resources, was accepted as a challenge in spite of the limited resources then available.

In 1968-1969 the intramural laboratories and offices were moved from temporary quarters to the new Building 37 on the NIH reservation. Unfortunately, at that time, when a major expansion in the programs was called for, the Carcinogenesis Program was hit by the reduction in positions that affected all Institutes, and the total position strength underwent a loss of about 20%. Only at the end of Fiscal Year 1971 did the position ceiling reach back to the 1968 level, and in Fiscal Year 1972 is raising to about 140. During these years, the Carcinogenesis staff gave invaluable cooperation to the task of substantially expanding the Carcinogenesis Program activities, both in-house and through contracts, at a time when a rapidly growing public awareness of the problems of environmental cancer hazards was giving a clear indication to the Nation of the increasing needs in this field.

In spite of the critical shortage of positions, by reassigning space, personnel and individual tasks and above all by the untiring efforts and cooperation of many members of the staff, several major new programs were activated.

A new Experimental Pathology Branch was established besides the previously existing Biology Branch and Chemistry Branch. Special units were set up in the Office of the Associate Scientific Director: a Program and Data Analysis Unit, a Lung Cancer Unit, and an Epidemiologic Pathology Unit (transferred from the Demography Program). In addition, the Registry of Experimental Cancers has been recently transferred administratively from the Office of the Director, NCI, to the Carcinogenesis Program. In order to provide an effective management structure to the contract-supported program, an Office of Collaborative Research Coordination was also established and eight segments were created to manage and coordinate the expanding contract program, which has presently reached a level of over $22 million.

B. *ORGANIZATION*

The organization of the Carcinogenesis Program is presently divided into two major components: Intramural Program and Collaborative Program. The

former is articulated in the Office of the Associate Scientific Director for Carcinogenesis with its units and in three branches, each with several sections and units. The latter is articulated into eight segments. In addition to these staff functions, several advisory groups provide scientific consultation and review.

The present organization includes the following components:

1. *INTRAMURAL PROGRAM*

The Office of the Associate Scientific Director for Carcinogenesis (U. Saffiotti, M.D., ASD; J. A. Cooper, II, Ph.D., Acting deputy ASD):

Epidemiologic Pathology Unit (J. Berg, M.D., Head)
Lung Cancer Unit (M. B. Sporn, M.D., Head)
Program and Data Analysis Unit (N. P. Page, D.V.M., Acting Head)

The Biology Branch (H. J. Rapp, Sc.D., Chief):

Cellular Immunity Section (B. Zbar, M.D., Head)
Cytogenetics and Cytology Section (J. A. DiPaolo, Ph.D., Head)
Immunochemistry Section (T. Borsos, Sc.D., Head)
Molecular Separations Unit(H. J. Rapp, Sc.D., Acting Head)

The Chemistry Branch (H. V. Gelboin, Ph.D., Chief):

Cell Growth Regulation Section (J. P. Bader, Ph.D., Head)
Molecular Carcinogenesis Section (H. V. Gelboin, Ph.D., Acting Head)
Nucleic Acids Section (C. W. Dingman, M.D., Head)
Protein Section (A. C. Peacock, Ph.D., Head)

The Experimental Pathology Branch (R. R. Bates, M.D., Chief):

Bioassay Section (R. R. Bates, M.D., Head)
Carcinogen Screening Section (J. H. Weisburger, Ph.D., Head)
Histopathology Unit (R. R. Bates, M.D., Acting Head)

2. *COLLABORATIVE PROGRAM*

The Office of the Associate Scientific Director for Carcinogenesis (U. Saffiotti, M.D., ASD; A. H. Heim, Ph.D., Coordinator of Collaborative Research)

Bioassay Segment (J. H. Weisburger, Ph.D., Director; R. F. Newell, Manager[1])
Biological Models Segment (R. R. Bates, M.D., Director; R. Pledger, Ph.D., Manager)
Biology and Immunology Segment (H. J. Rapp, Sc.D., Director; A. H. Heim, Ph.D., Manager)

[1] Served as Segment Manager until his retirement on March 17, 1972.

Chemistry and Molecular Carcinogenesis Segment (A. C. Peacock, Ph.D., Director; R. Pledger, Ph.D., Manager)
Colon Cancer Segment (J. Berg, M.D., Director; S. Silverman, Ph.D., Manager)
Information and Resources Segment (J. A. Cooper, II, Ph.D., Director; S. Siegel, Ph.D., Manager)
Lung Cancer Segment (M. Sporn, M.D., Director; C. Smith, Ph.D., Manager)
Tobacco Research Segment (G. Gori, Ph.D., Director; T. Owen, Ph.D., Manager)

3. *ADVISORY GROUPS AND CONSULTANTS*

a. *Carcinogenesis Advisory Panel*

Dr. Emmanuel Farber, Professor of Pathology, Fels Research Institute, Temple University, Philadelphia, Pennsylvania

Dr. Theodore Hauschka, Director of Cancer Research, Roswell Park Memorial Institute, Buffalo, New York

Dr. Maureen Henderson, Professor and Chairman, Preventive Medicine and Rehabilitation, University of Maryland, Baltimore, Maryland

Dr. Paul Kotin, Vice-President, Health Sciences Center, Temple University, Philadelphia, Pennsylvania

Dr. Peter Magee, Professor of Biochemistry, Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, England

Dr. James Miller, Professor of Oncology, McArdle Memorial Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin

Dr. Norton Nelson, Director, Institute of Environmental Medicine, New York University Medical Center, New York, New York

Dr. James Price, Vice President, Experimental Therapy, Abbott Laboratories, North Chicago, Illinois

Dr. Arthur Upton, Dean, School of Basic Health Sciences, State University of New York, Stony Brook, New York

Dr. Gerald Wogan, Professor of Food Toxicology, Massachusetts Institute of Technology, Cambridge, Massachusetts

b. *Carcinogenesis Contract Program Management Group*

Dr. Umberto Saffiotti, Associate Scientific Director for Carcinogenesis, *Chairman*

Dr. Allen Heim, Coordinator for Collaborative Research, *Executive Secretary* [2]

Dr. Richard Bates, Director, Biological Models Segment

Dr. John Berg, Director, Colon Cancer Segment

Dr. John Cooper, Director, Information and Resources Segment

Dr. Thaddeus Domanski, Program Director for Carcinogenesis, Extramural Activities, NCI

Dr. Harry Gelboin, Chief, Chemistry Branch, Carcinogenesis

Dr. Gio Gori, Director, Tobacco Research Segment

Dr. Andrew Peacock, Director, Chemistry and Molecular Carcinogenesis Segment

Dr. Herbert Rapp, Director, Biology and Immunology Segment

Dr. Michael Sporn, Director, Lung Cancer Segment

Dr. John Weisburger, Director, Bioassay Segment

c. *Segment Advisory Groups*

(1) Bioassay Segment

Dr. John Weisburger, *Director*

Mr. Raymond Newell, *Manager*[2]

Dr. Richard Adamson, Head, Pharmacology and Experimental Therapeutics Section, GL&C, NCI

Dr. William Deichmann, Director, Research and Teaching Center of Toxicology, University of Miami, Coral Gables, Florida

Dr. Leo Friedman, Director, Division of Toxicology, Bureau of Foods, Food and Drug Administration, Washington D.C.

Dr. Harold Grice, Head, Pathology Toxicology Section, Food and Drug Directorate, Ottawa, Canada

Dr. Paul Harris, 4114 East 65th Street, Indianapolis, Indiana

Dr. Charles Irving, Chief, Cancer Research Laboratory, Veterans Administration Hospital, Memphis, Tennessee

Dr. Gerhard Krueger, Laboratory of Pathology, GL&C, NCI

[2] Mr. Raymond Newell served as Executive Secretary of the Carcinogenesis Contract Program Management Group and as Segment Manager of the Bioassay Segment until his retirement on March 17, 1972.

Mr. Samuel Poiley, Head, Mammalian Genetics and Animal Production Section, Chemotherapy, NCI

Dr. Lionel Poirier, Carcinogen Screening Section, Experimental Pathology Branch, Carcinogenesis, Etiology, NCI

Dr. William Priester, Head, Epizoology Section, Epidemiology Branch, Demography, Etiology, NCI

Dr. Harry Wood, Chief, Drug Development Branch, Chemotherapy, NCI

(2) Biological Models Segment

Dr. Richard Bates, *Director*

Dr. Richard Pledger, *Manager*

Dr. John Berg, Head, Epidemiologic Pathology Unit, Carcinogenesis, Etiology, NCI

Dr. Howard Bern, Professor of Zoology, Department of Zoology, University of California, Berkeley, California

Dr. Thomas Dao, Chief, Department of Breast Surgery and Endocrine Research Laboratory, Roswell Park Memorial Institute, Buffalo, New York

Dr. Pietro Gullino, Head, Tumor Physiopathology Section, Laboratory of Biochemistry, GL&C, NCI

Dr. Elizabeth Miller, Professor of Oncology, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin

Dr. Jerry Rice, Experimental Pathology Branch, Carcinogenesis, Etiology, NCI

Dr. Edward Smuckler, Professor of Pathology, Department of Pathology, University of Washington, Seattle, Washington

Dr. Michael Sporn, Head, Lung Cancer Unit, Carcinogenesis, Etiology, NCI

Dr. Benjamin Trump, Chairman, Department of Pathology, University of Maryland, Baltimore, Maryland

Dr. Paul Webster, Chief, Gastroenterology Division, Medical College of Georgia, Augusta, Georgia

(3) Biology and Immunology Segment

Dr. Herbert Rapp, *Director*

Dr. Allen Heim, *Acting Manager*

Dr. Tibor Borsos, Head, Immunochemistry Section, Biology Branch, Carcinogenesis, Etiology, NCI

Dr. Joseph DiPaolo, Head, Cytogenetics and Cytology Section, Biology Branch, Carcinogenesis, Etiology, NCI

Dr. Michael Edidin, Department of Biology, Johns Hopkins University, Baltimore, Maryland

Dr. Hugues Ryser, Professor, Department of Cell Biology and Pharmacology, University of Maryland, Baltimore, Maryland

Dr. Paul Ts'o, Professor of Biophysical Chemistry, Department of Radiological Science, Johns Hopkins University, Baltimore, Maryland

Dr. Willie Turner, Professor and Chairman, Department of Microbiology, Howard University, Washington D.C.

Dr. Berton Zbar, Head, Cellular Immunity Section, Biology Branch, Carcinogenesis, Etiology, NCI

(4) Chemistry and Molecular Carcinogenesis Segment

Dr. Andrew Peacock, *Director*

Dr. Richard Pledger, *Manager*

Dr. John Bader, Head, Cell Growth Regulation Section, Chemistry Branch, Carcinogenesis, Etiology, NCI

Dr. Donald Brown, Staff Member, Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland

Dr. Wesley Dingman, Head, Nucleic Acids Section, Chemistry Branch, Carcinogenesis, Etiology, NCI

Dr. Harry Gelboin, Chief, Chemistry Branch, Carcinogenesis, Etiology, NCI

Dr. Marie Green, Protein Section, Chemistry Branch, Carcinogenesis, Etiology, NCI

Dr. Dolph Hatfield, Cell Growth Regulation Section, Chemistry Branch, Carcinogenesis, Etiology, NCI

Dr. Robert Holley, Fellow, Salk Institute for Biological Studies, San Diego, California

Dr. Paul Howard-Flanders, Professor of Radiobiology and Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut

Dr. Tsuyoshi Kakefuda, Nucleic Acids Section, Chemistry Branch, Carcinogenesis, Etiology, NCI

Dr. Arthur Ness, Protein Section, Chemistry Branch, Carcinogenesis, Etiology, NCI

Dr. Melvin Newman, Professor of Chemistry, Department of Chemistry, Ohio State University, Columbus, Ohio

Dr. Hewson Swift, Professor of Biology, University of Chicago, Chicago, Illinois

(5) Colon Cancer Segment

Dr. John Berg, *Director*

Dr. Sidney Silverman, *Manager*

Dr. Thomas Almy, Professor and Chairman, Department of Medicine, Dartmouth Medical School, Hanover, New Hampshire

Dr. Richard Bates, Chief, Experimental Pathology Branch, Carcinogenesis, Etiology, NCI

Dr. Cecile Edwards, Chairman, Department of Home Economics, Howard University, Washington D.C.

Dr. Gary Glober, Resident Investigator for Biometry Branch, Demography Program, NCI, Kuakini Hospital and Home, Honolulu, Hawaii

Dr. Edward High, Professor and Chairman, Department of Biochemistry, Meharry Medical College, Nashville, Tennessee

Dr. Alan Hofmann, Professor of Medicine and Physiology, Mayo Clinic and Foundation, Rochester, Minnesota

Dr. Reino Kallio, Professor of Microbiology, University of Illinois, Urbana, Illinois

Dr. Gert Laqueur, Chief, Laboratory of Experimental Pathology, NIAMD

Dr. James Miller, Professor of Oncology, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin

Dr. John Weisburger, Head, Carcinogen Screening Section, Experimental Pathology Branch, Carcinogenesis, Etiology, NCI

(6) Information and Resources Segment

Dr. John Cooper, II, *Director*

Dr. Sidney Siegel, *Manager*

Dr. Robert Flynn, Assistant Director of Animal Facilities, Argonne National Laboratory, Argonne, Illinois

Dr. Don Gibson, Health Scientist Administrator, Aging Branch, NICHD

Dr. Madeline Henderson, Science Fellow, Department of Commerce, Washington D.C.

Dr. David Kaufman, Lung Cancer Unit, Carcinogenesis, Etiology, NCI

Dr. Larry Keefer, Office of the Associate Scientific Director for Carcinogenesis, Etiology, NCI

Mr. Terence Kuch, Automatic Data Processing Management Section, Biometry Branch, Demography, Etiology, NCI

Dr. Norbert Page, Acting Head, Program and Data Analysis Unit, Carcinogenesis, Etiology, NCI

Dr. Eugene Sawicki, Chief, Laboratory Measurements Research Section, Division of Chemistry and Physics, Environmental Protection Agency, Research Triangle Park, North Carolina

Dr. Charles Warner, Organic Analytical Chemist, Ayerst Laboratories, Rouses Point, New York

Dr. Elizabeth Weisburger, Carcinogen Screening Section, Experimental Pathology Branch, Carcinogenesis, Etiology, NCI

Dr. Isaac Welt, Director of Scientific and Technical Information System Program, American University, Washington D.C.

(7) Lung Cancer Segment

Dr. Michael Sporn, *Director*

Dr. Carl Smith, *Manager*

Dr. Richard Bates, Chief, Experimental Pathology Branch, Carcinogenesis, Etiology, NCI

Dr. John Berg, Head, Epidemiologic Pathology Unit, Carcinogenesis, Etiology, NCI

Dr. Roswell Boutwell, Professor of Oncology, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin

Dr. Jacob Churg, Professor of Pathology, Mount Sinai Hospital, New York, New York

Dr. John Cooper, II, Acting deputy Associate Scientific Director for Carcinogenesis, Etiology, NCI

Dr. Luigi DeLuca, Lung Cancer Unit, Carcinogenesis, Etiology, NCI

Dr. Robert Drew, Pharmacology and Toxicology Branch, NIEHS, Research Triangle Park, North Carolina

Dr. Emmanuel Farber, Professor of Pathology, Fels Research Institute, Philadelphia, Pennsylvania

Dr. Hermann Lisco, Associate Professor of Anatomy, Department of Anatomy, Harvard Medical School, Boston, Massachusetts

Dr. Sergei Sorokin, Assistant Professor of Anatomy, Department of Physiology, Harvard School of Public Health, Cambridge, Massachusetts

Dr. I. Bernard Weinstein, Associate Professor of Medicine, Columbia University College of Physicians and Surgeons, New York, New York

(8) Tobacco Research Segment

Dr. Gio Gori, *Director*

Dr. Thomas Owen, *Manager*

Dr. William Bates, Director, Research Department, Liggett and Myers, Inc, Durham, North Carolina

Dr. Fred Bock, Director, Orchard Park Laboratories, Roswell Park Memorial Institute, Orchard Park, New York

Dr. Roswell Boutwell, Professor of Oncology, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin

Dr. Michael Guerin, Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Dr. I. W. Hughes, Director, Research and Development, Brown and Williamson Tobacco Corp., Louisville, Kentucky

Dr. Charles Kensler, Senior Vice President, Arthur D. Little, Inc., Cambridge, Massachusetts

Dr. Gardner McMillan, Chief, Arteriosclerotic Disease Branch, NHLI

Dr. Umberto Saffiotti, Associate Scientific Director for Carcinogenesis, Etiology, NCI

Dr. Marvin Schneiderman, Associate Scientific Director for Demography, Etiology, NCI

Dr. Irving J. Selikoff, Director, Division of Environmental Medicine, Mount Sinai School of Medicine, New York, New York

Dr. Murray Senkus, Director of Research, R. J. Reynolds Tobacco Company, Winston-Salem, North Carolina

Dr. A. W. Spears, Director, Research and Development, P. Lorillard Research Center, Greensboro, North Carolina

Dr. T. C. Tso, Plant Physiologist Leader, Tobacco Quality Investigations, Agriculture Research Service, Beltsville, Maryland

Dr. Benjamin Van Duuren, Professor of Environmental Medicine, New York University Medical Center, New York, New York

Dr. Helmut Wakeham, Vice-President, Corporate Research and Development, Philip Morris, Inc. Richmond, Virginia

Dr. Ernest Wynder, President, American Health Foundation, New York, New York

d. *Other Consultants*

Dr. Charles Apffel, Pondville Hospital, Walepole, Massachusetts

Dr. Claudio Basilico, New York University, New York, New York

Dr. Thomas Brent, McGill University, Cancer Research Unit, Montreal Canada

Mr. Donald Buchsbaum, 85 Green Moor Way, Henrietta, New York

Mr. Carter Cook, University of Illinois, Urbana, Illinois

Dr. M. M. Coombs, Imperial Cancer Research Foundation, London, England

Dr. Pierre DeCoufle, National Institute of Occupational Safety and Health, Rockville, Maryland

Dr. Nicholas DiLuzio, Tulane University, New Orleans, Louisiana

Dr. John Dyminski, Syracuse University, Syracuse, New York

Dr. Kjell Elgjo, University of Oslo, Oslo, Norway

Dr. John Epstein, University of California, San Francisco, California

Dr. Donald Everson, Union Carbide Corporation, Tarrytown, New York

Dr. Lawrence Fishbein, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

Dr. Mariam Fukami, Temple University, Philadelphia, Pennsylvania

Dr. Frank Hemming, University of Liverpool, Liverpool, England

Dr. Dietrich Hoffman, American Health Foundation, New York, New York

Dr. James Innes, 4621 Langdrum Lane, Chevy Chase, Maryland

Dr. Olav Iversen, University of Oslo, Oslo, Norway

Dr. William Jones, III, Southern Illinois University, Carbondale, Illinois

Dr. C. Y. Kang, Department of Biology, McMaster University, Hamilton, Ontario, Canada

Dr. Herman Kraybill, Food and Drug Administration, Washington D.C.

Dr. Robert Kroes, 7105 Exeter Road, Bethesda, Maryland

Dr. Michael Lieberman, Fels Research Institute, Philadelphia, Pennsylvania

Dr. William Lijinsky, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Dr. Robert Love, Jefferson Medical College, Philadelphia, Pennsylvania

Dr. Toni Mariani, University of Minnesota, Minneapolis, Minnesota

Dr. Bernard McNamara, Edgewood Arsenal, Edgewood, Maryland

Dr. Gerald Medoff, Washington University Medical School, St. Louis, Missouri

Dr. Daniel Menzel, Duke University Medical School, Durham, North Carolina

Dr. Gail Miller, Thomas Jefferson University, Philadelphia, Pennsylvania

Dr. Paul Newberne, Massachusetts Institute of Technology, Cambridge, Massachusetts

Dr. David Nelson, Department of Nutrition and Food Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts

Dr. Thomas Osdene, Philip Morris Research Center, Richmond, Virginia

Dr. Jerome Pomeranz, Cleveland Metropolitan General Hospital, Cleveland, Ohio

Dr. Ronald Raineri, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin

Dr. Josef Riman, Czechoslovak Academy of Science, Praha, Czechoslovakia

Dr. Peter Roller, University of Virginia, Charlottesville, Virginia

Mr. Howard Rosenberg, 577 14th Avenue, San Francisco, California

Dr. Verald Rowe, Dow Chemical Company, Midland, Michigan

Dr. Elizabeth Russell, Jackson Laboratories, Bar Harbor, Maine

Dr. Geno Saccomanno, St. Mary's Hospital, Grand Junction, Colorado

Dr. James Selkirk, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin

Dr. Gary Stein, Temple University, Philadelphia, Pennsylvania

Dr. D. Stolz, Food and Drug Directorate, Ottawa, Canada

Dr. Gary Stoner, University of California, San Diego, California

Dr. T. Sugimura, National Cancer Research Institute, Tokyo, Japan

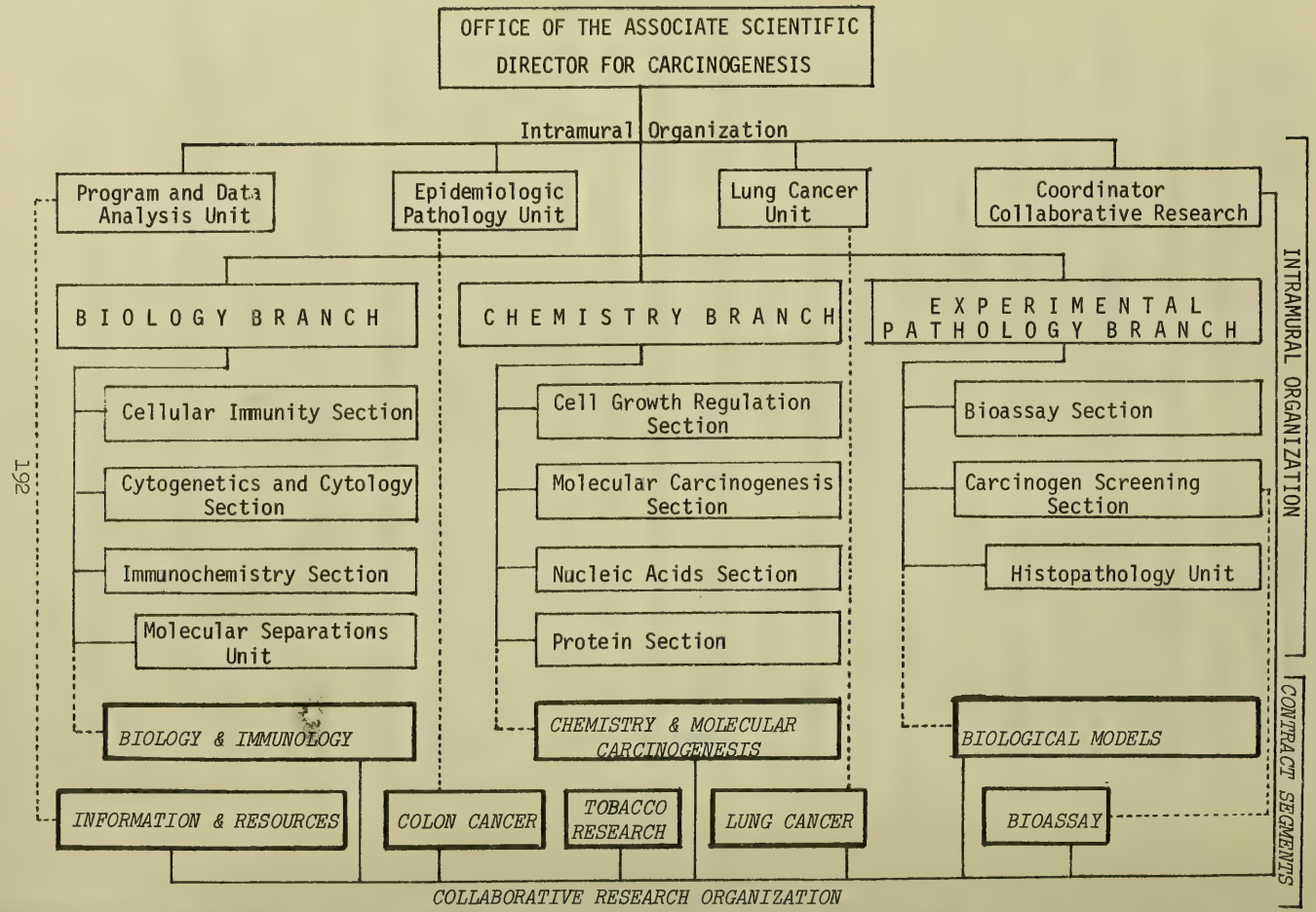
Dr. Bela Toth, University of Nebraska, The Eppley Institute for Research on Cancer, Omaha, Nebraska

Dr. Marcel Waks, Biological Sciences Department, Columbia University, New York, New York

Dr. David Wilkinson, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin

Dr. C. Y. Yang, Department of Biology, McMaster University, Hamilton, Ontario, Canada

Dr. Stuart Yuspa, 50th and Wynnefield Avenues, Philadelphia, Pennsylvania


OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR FOR CARCINOGENESIS
Intramural Organization
Program and Data Analysis Unit
Epidemiologic Pathology Unit
Lung Cancer Unit
Coordinator Collaborative Research
BIOLOGY BRANCH
CHEMISTRY BRANCH
EXPERIMENTAL PATHOLOGY BRANCH
Cellular Immunity Section
Cytogenetics and Cytology Section
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Molecular Separations Unit
Cell Growth Regulation Section
Molecular Carcinogenesis Section
Nucleic Acids Section
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Carcinogen Screening Section
Histopathology Unit
BIOLOGY & IMMUNOLOGY
CHEMISTRY & MOLECULAR CARCINOGENESIS
BIOLOGICAL MODELS
INFORMATION & RESOURCES
COLON CANCER
TOBACCO RESEARCH
LUNG CANCER
BIOASSAY
COLLABORATIVE RESEARCH ORGANIZATION
INTRAMURAL ORGANIZATION
CONTRACT SEGMENTS

In Fiscal Year 1972 the major organizational change was represented by the establishment of the eight segments of the collaborative program and by the appointment of several advisory groups. Each segment is directed by a senior member of the Carcinogenesis staff, assisted by a segment manager. Other members of the staff serve as project officers on individual contracts.

The *Carcinogenesis Advisory Panel* has been appointed to provide overall discussion and advice to the Carcinogenesis Program staff on the development of the program and its priorities in the context of future activities of the National Cancer Institute.

The *Carcinogenesis Contract Program Management Group* has been established to provide review for need, priority and relevance for the whole program.

The *Segment Advisory Groups* has been established to provide advice on program needs and priorities as well as technical review of contract proposals.

The *Carcinogenesis Intramural Program Group* provides a coordinating and planning function at the senior staff level. The entire professional staff is now essentially participating in the planning and monitoring of the overall program, providing a vital correlation between intramural and collaborative research programs.

Close liaison will continue to be maintained with the Etiology Program Advisory Committee at the Etiology Area level, and is hoped that an effective liaison will be established with the new National Cancer Advisory Board.

Other organizational changes are based on the capability for additional recruitment that was finally made possible by the increased appropriation in Fiscal Year 1972. Top priority was given to the recruitment of competent science managers fully devoted to the coordination of scientific management for the segments. An experienced analytical chemist was recruited to establish an Analytical Chemistry Unit and provide a much needed focal point in the methodologies for chemical identification of carcinogens. Recruitment of a veterinary pathologist is under way to extend the present activity of the Registry of Experimental Cancers and establish a much needed national reference center for laboratory animal tumors used as end point in the evaluation of carcinogens. This Registry has been functioning under the leadership of Dr. H. L. Stewart, former chief, Laboratory of Pathology, GL&C, and now consultant to NCI.

A major new organizational group has been requested (recruitment action presently pending): it will create an Office of Environmental Carcinogenesis directly reporting to the ASD. It will be headed by a senior scientist with extensive experience in environmental carcinogen evaluation and will be staffed with experts in the fields of food additives, pesticides, drugs, air and water pollution, industrial and occupational exposures, natural products, and tobacco. This group of experts will provide documentation, identify needs and priorities, and essentially provide a liaison between the field of carcinogenesis, with its rapid methodological and scientific progress, and their respective areas of environmental relevance. A particular task of this Office will be that of developing a closer collaboration with other Government agencies and with industry.

Further recruitment is under way to provide adequate staff support to the pressing task of expanding and monitoring the bioassay program, including standardized protocols, data handling system, resources, and biometric evaluation of results.

Further recruitment is also under way to provide key competence in the Experimental Pathology Branch to implement its commitment to the development of organ-oriented biological models, particularly in the areas of endocrine-related cancers, pancreas, breast and skin cancers.

In Fiscal Year 1972, as in previous years, the Associate Scientific Director for Program, Etiology (Dr. G. B. Gori), has made an outstanding contribution to the Carcinogenesis Program, serving as project officer on several important contracts in Carcinogenesis. He has also accepted the responsibility of serving as Segment Director for the Tobacco Research Segment and Chairman of its advisory panel, the Tobacco Working Group.

C. *MANAGEMENT ACTIVITIES*

A science-based management system is essential for the effective management of the increasingly complex contract-supported collaborative program. Last year a goal was set to establish a management staff and separate advisory groups for each specific, major program areas and for the Carcinogenesis Program in general. This objective has been achieved with the following results: eight program areas designated as segments have been established. Senior scientists and staff of the intramural research programs provided the operating nucleus of each segment, thus ensuring a sound scientific base for the contract program. Segment directors who provide the scientific leadership and project officers who provide the constant technical guidance and operational direction to each program area are members of the intramural staff.

Just as the technical aspects of the Carcinogenesis Collaborative Program are built upon a staff having expertise, research experience, and competence in particular areas, so too the management of the Collaborative Program is built upon scientist-administrators with both research and administrative experience. These scientist-administrators have been designated as segment managers and provide management support to the segment director, project officers, and outside advisors. The eight segments are as follows: Bioassay, Biological Models, Biology and Immunology, Chemistry and Molecular Carcinogenesis, Colon Cancer, Information and Resources, Lung Cancer, and Tobacco Research.

Each segment has an advisory group made up of the segment director as chairman, the segment manager as executive secretary, senior intramural staff, and at least five advisors drawn from the scientific community. The advisory group either in its entirety or as subgroups participates in segment planning, preparation of requests for competitive proposals, review of proposals and monitoring of contracts.

All of the segments have established this basic management structure during Fiscal Year 1972. The Lung Cancer Segment has held a contractors' conference involving all of its contractors as well as the staff and outside advisors. A general contractors' conference is presently planned for the whole Carcinogenesis Program in the Fall of 1972.

Each segment has the responsibility for developing its own specific program within the framework of the overall Carcinogenesis Program, in keeping with the fundamental objectives of the segment. As a basis for the implementation of its operational plans, each segment receives a preliminary budget apportionment from the Carcinogenesis budget. While this is an essential factor to good planning and program management, it also represents the realistic limiting factor requiring some difficult priority choices between continuing or expanding existing programs and initiating new ones. When new ones are initiated, requests for proposals are formulated at the segment level and proposals are reviewed by the segment for technical merit and relevance to the segment program. Recommendations for support of a particular research program and establishment of a contract is made by the segment to the Carcinogenesis Contract Program Management Group (CCPMG).

The CCPMG is an advisory group which includes all segment directors and serves the following functions. It provides review of proposals for need, priority, and relevance within the total Carcinogenesis Program, it functions in an advisory capacity to the Associate Scientific Director for Carcinogenesis, and it reviews "Requests for Proposals" (RFPs) before they are advertised for competitive proposals. This last function is designed to prevent the initiation of inadequately structured RFPs and more importantly, to prevent the initiation of a request for work which may be considered to be of low relevance and priority to the overall program. Membership of the CCPMG is as follows: The Associate Scientific Director for Carcinogenesis (Chairman), the Coordinator for Collaborative Research (Executive Secretary[3]), all of the segment directors and branch chiefs, and the Program Director for Carcinogenesis from the Extramural Activities Area (to provide liaison with the Grants Program). Segment managers, contract specialists, administrative officers, and appropriate project officers attend the meetings as observers and resource staff. The membership and resource staff of this senior advisory group represents all of the major elements in Carcinogenesis: The Office of the Associate Scientific Director for Carcinogenesis, intramural scientific staff, collaborative program scientific and management staff, contract specialists, and Carcinogenesis' administrative staff, as well as the representative from the Grants Area.

The contract development and review process, as presently functioning for the Carcinogenesis Program, is outlined in the following chart.

Following the completion of the dual review process, final recommendation on priority and funding is made by the Associate Scientific Director for Carcinogenesis to the Scientific Director for Etiology.

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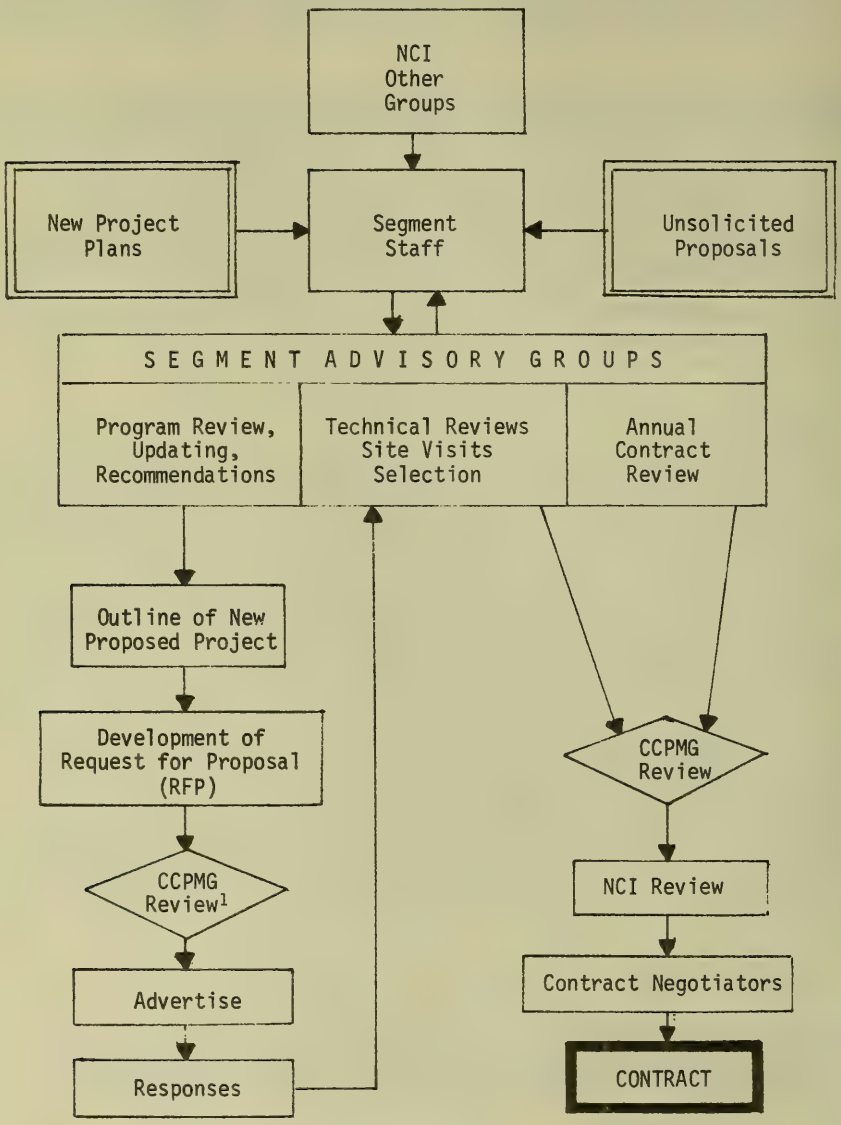
[3] Until March 1972 the Coordinator for Collaborative Research was a member and there was a separate Executive Secretary.

CARCINOGENESIS CONTRACT DEVELOPMENT AND REVIEW PROCESS



[1]CCPMG - Carcinogenesis Contract Program Management Group

The contract specialists (Mr. M. Fortin and Mr. L. Tidmore) assigned to this Program from the Etiology Section of the Research Contracts Branch, OD, provided coordination between scientific management and administrative mechanisms of negotiation, award and fiscal monitoring of contracts. They performed an essential and most effective function for the implementation of the complex research programs in the Area.

D. *PROGRAM PLANS*

The overall plan for the Carcinogenesis Program was laid down in 1968 and has served as a solid basis for the implementation of new efforts, as a guide for the expansion of the Program and as a reference for its monitoring. Particular emphasis is now given to the development of special program on the main organ-specific types of cancers, as seen in man. The first of these to be implemented was the Special Lung Cancer Program, developed in Fiscal Year 1969, partly by the Lung Cancer Task Force and by the Tobacco Working Group. Its general structure was patterned on that of the general Carcinogenesis Plan. An intramural Lung Cancer Unit was established to serve as a research focus in this effort and to provide strong correlation between staff and contractors.

A new plan has been developed on the etiology of colon cancer, through an ad hoc group chaired by Dr. J. W. Berg, with the collaboration of several staff members in close liaison with the National Task Force on Colon Cancer. A contract program has been established in this important area, according to the new plan.

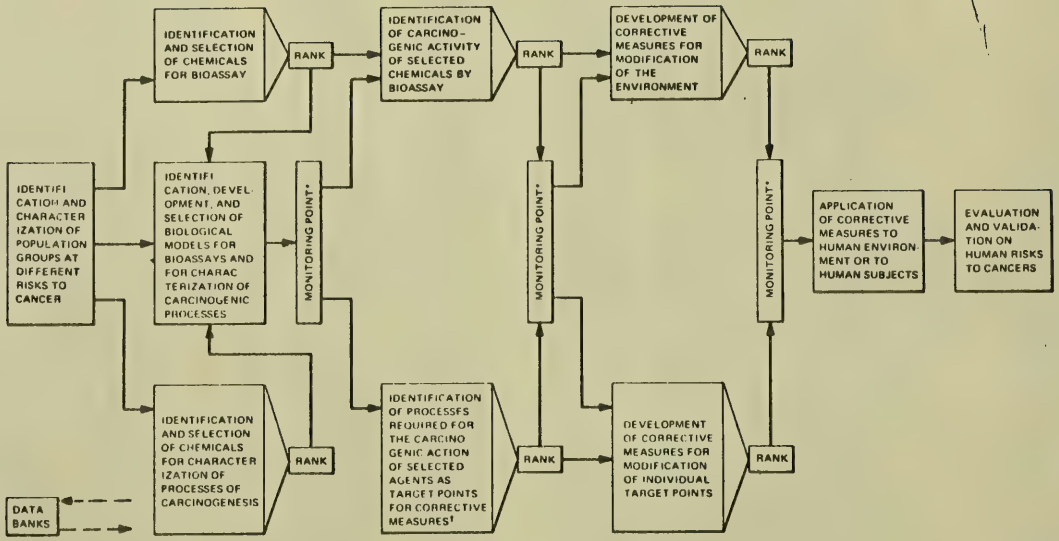
Preliminary steps have been taken towards the development of special programs on childhood cancers, on bladder cancer and on cancer of the pancreas, again in close liaison with national efforts in these fields.

In response to the planning needs for the development of the new Cancer Conquest Program, the Carcinogenesis staff has outlined a five-year plan and developed a number of supporting documents, including construction plans and plans for the utilization of research facilities at Fort Detrick.

A summary of the general Carcinogenesis Plan is given below.

Program Plan on Chemical Carcinogenesis and Prevention of Cancers

The plan was developed in 1968 through an extensive series of planning sessions conducted by Dr. Carl G. Baker, then Scientific Director for Etiology, Dr. Umberto Saffiotti, Associate Scientific Director for Carcinogenesis, and Mr. Louis M. Carrese, with the participation of Dr. Abraham Cantarow, and Mr. Richard A. Turbelic. The Report of the Discussion Groups on Chemical Carcinogenesis, coordinated by Dr. Cantarow for the National Advisory Cancer Council, served as an excellent basis for outlining the research needs in the field of carcinogenesis. The convergence technique was used to identify the major objectives and the linear array of subsequent phases leading to their implementation. An outline of the program plan is attached (see Chart) and its main phases are discussed below. The program is articulated in a number of segments. The first segment deals with (a) the epidemiologic identification


IDENTIFICATION AND SELECTION OF CHEMICALS FOR BIOASSAY
RANK
IDENTIFICATION OF CARCINO-GENIC ACTIVITY OF SELECTED CHEMICALS BY BIOASSAY
RANK
DEVELOPMENT OF CORRECTIVE MEASURES FOR MODIFICATION OF THE ENVIRONMENT
RANK
IDENTIFI CATION AND CHARACTER IZATION OF POPULATION GROUPS AT DIFFERENT RISKS TO CANCER
IDENTIFI CATION, DEVEL-OPMENT, AND SELECTION OF BIOLOGICAL MODELS FOR BIOASSAYS AND FOR CHARAC TERIZATION OF CARCINOGENIC PROCESSES
MONITORING POINT*
MONITORING POINT*
MONITORING POINT*
APPLICATION OF CORRECTIVE MEASURES TO HUMAN ENVIRON-MENT OR TO HUMAN SUBJECTS
EVALUATION AND VALIDA-TION ON HUMAN RISKS TO CANCERS
IDENTIFICATION AND SELECTION OF CHEMICALS FOR CHARACTER IZATION OF PROCESSES OF CARCINOGENESIS
RANK
IDENTIFICATION OF PROCESSES REQUIRED FOR THE CARCINO GENIC ACTION OF SELECTED AGENTS AS TARGET POINTS FOR CORRECTIVE MEASURES†
RANK
DEVELOPMENT OF CORRECTIVE MEASURES FOR MODIFICATION OF INDIVIDUAL TARGET POINTS
RANK
DATA BANKS

*Monitoring points: According to program relevance and needs, determine further action and channel through next phases

†Through studies on the penetration of the agents into the organism, their transport, retention, and elimination, their metabolic conversion to the proximate carcinogen, the cellular and tissue factors required for such processes, the penetration into target cells and interaction with cell constituents, the neoplastic transformation, and the factors required for the growth of transformed cells into tumors

CHEMICAL CARCINOGENESIS AND PREVENTION OF CANCER (SUMMARY OF PROGRAM PLANS).

of population groups showing different risks to different types of cancer and (b) to the characterization of such individuals as to their environmental factors and their biological and functional parameters. The program then develops the following distinct approaches:

(1) identification of carcinogenic activity of selected chemicals by bioassay,

(2) identification, development, and selection of biological models for carcinogenesis bioassays as well as for the characterization of carcinogenesis processes,

(3) identification of processes required for the carcinogenic action of selected agents as target points for corrective measures.

The first approach requires an initial phase devoted to the identification and selection of chemical agents to be entered in the bioassay systems. The development and implementation of bioassay procedures will require a great deal of accurate standardization so that selected chemical agents will be tested through a battery of precisely defined and reproducible bioassay systems, each well characterized for its sensitivity to specific carcinogenic effects.

After appropriate monitoring for relevance and for program needs, the results of the first phases of the program will lead to the development of corrective measures based, on one hand, on modifications of the environment and, on the other hand, based on modifications of individual target points in the process of carcinogenesis, identified by previous studies. These target points can be visualized as taking place at any one of the several steps required for the process of carcinogenesis to be completed. They include the following: (a) penetration of the chemical agent into the whole organism; (b) transport, retention and elimination of the chemicals in tissues (molecular logistics); (c) metabolic pathways to proximate carcinogen; (d) cell and tissue factors required for the previous steps; (e) penetration into target cells and interaction with cell constituents; (f) neoplastic transformation and conditioning factors; and (g) growth regulation of transformed cells.

The results thus obtained will be in turn appropriately monitored for relevance; the program then considers the development of corrective measures and ultimately suggests application of such corrective measures through the human environment or through the human subject. The last phase of the program deals with the evaluation and validation of the results of such corrective measures in the human situation and is expected to lead to the recognition of decreased cancer risks and in the populations originally studied in Phase I. Data banks will be organized for each of the major phases of the program and they will be a necessary source of information to be used for the monitoring of the whole program.

The program is aimed at a comprehensive attack to the problem of chemical carcinogenesis. It recognizes the need for very extensive bioassay efforts of an order of magnitude much greater than that presently available, but also recognizes that the accumulation of information on the carcinogenicity

of chemicals in animal systems will not provide a direct extrapolation to the hazards in man. Moreover, it is predictable that a large number of chemical carcinogens will be difficult to remove and, therefore, will remain in the environment of man for a long time to come even after being identified (e.g. combustion products, cigarette smoke, natural food contaminants such as mycotoxins, metals and other air pollutants). It is, therefore, imperative that an effort be made not only to identify and possibly remove an increasing number of carcinogens from the environment, but also, and concurrently, to identify steps in their mode of penetration into the organisms and the target tissues and in their critical interaction with cell constituents so that these steps could be exploited as target points for inhibitory or protective measures. The considerable development of refined technical methods presently available for the identification of these critical steps in carcinogenesis by studies in analytical chemistry, biochemistry, cell biology, molecular biology, and immunology can now be brought to bear on our ability to develop useful, protective measures against the ultimate effects of the carcinogenic process. The identification of critical parameters required for the carcinogenic action of certain groups of chemicals, obtained in animal studies, also represents the only direct link that can be applied to the study of the susceptibility of man to comparable exposure conditions.

For example, the establishment of a strong correlation between the enzymatic activation of certain chemicals to the proximate carcinogen by means of given groups of enzymes, such as the microsomal aryl hydroxylase for polynuclear hydrocarbons may lead to the identification of groups of individuals as being highly susceptible to certain specific carcinogenic exposures, pharmacologic inhibition of some of these enzyme systems may lead to a marked decrease of the cancer risk for such individuals. As other examples, analogous approaches can be made at the level of the control of protein synthesis in differentiation-dependent carcinogenic mechanisms (e.g. hormone-dependent tumors and vitamin A-dependent tumors) and at the level of the immunological control of the growth of transformed cells. A broad front of attack can thus be developed against the sequence of events required for the carcinogenic process to lead to the establishment of progressively growing invasive tumors.

The effective development of such studies depends in part on the availability of appropriate animal models, capable of reproducing tumor types analogous to those observed in man. The development of appropriate biological models is particularly important for the establishment of selectively sensitive bioassay systems. An important phase of the program consists in the selection and standardization of bioassay systems that could be reproduced in several laboratories under strictly controlled standardized conditions. This is an essential requirement if we want to collect and analyze large series of data on carcinogenesis tests. For this purpose, a detailed plan is being worked out for a data retrieval and analysis system for carcinogenesis studies. The present overall Program Plan in Chemical Carcinogenesis and Prevention of Cancers constitutes the basis for the development of detailed operational plans aimed at the implementation of each of its phases.

E. *SCIENTIFIC ACTIVITIES: PROGRESS HIGHLIGHTS*

The progress accomplished in the Carcinogenesis Area in Fiscal Year 1971 covers a broad front of attack to the problem of the identification of causative factors and the prevention of cancers. Many of the projects in this Area have been underway for several years and represent a patient, intensive, and costly long-term effort towards the attainment of our specific program goals. Work in chemical carcinogenesis is characterized to a large extent by the two-three year duration of each lifespan study in common laboratory rodents, with the resulting long-range commitment of efforts and resources. Other projects represent a complex effort to develop and refine the research methods necessary for studying the spectrum of chemical, biochemical, biological, and molecular mechanisms that are responsible for the host response to chemical and physical carcinogens, and for the identification of exploitable preventive measures.

Summary reports of the Office of the Associate Scientific Director, of the branches, and of the segments, as well as detailed reports of individual projects and contracts, will be presented in the following sections of this Annual Report. The highlights of these reports are summarized here in the perspective of the overall plan of operation of the Carcinogenesis Area. The Program Plan in Chemical Carcinogenesis, developed in Fiscal Year 1969, was the basis for the establishment of new projects. Most of the long-term projects that were underway before the Plan was implemented were brought to an orderly conclusion; therefore, only a few of the ongoing projects and contracts are only indirectly related to the Plan's design.

The following summary of progress highlights was prepared, for the convenience of the reader, as concise statements of some important areas of progress within the Carcinogenesis Program.

Since 1968 the operation of the Program has been developed along the lines designated in its Program Plan. The major accomplishments over the last four years have been obtained in the following areas of high priority:

Carcinogenesis bioassays of environmental chemicals under a single *standardized* feeding protocol in rats and mice. This program is currently operated through nine laboratories under contract and includes tests of 148 compounds in 80,000 animals. A major expansion of this resource is underway in the present fiscal year. Ten other contracts use a variety of other specialized test systems for special groups of compounds.

A computerized Bioassay Data System has been designed and tried out and is now operative in a majority of the bioassay contracts. It provides, for the first time, an effective centralized monitoring resource and a basis for data analysis.

Several information and resources systems have been set up, including the major first attempt to collect information on levels of exposure to environmental chemicals from all sources as a basis for priority ranking in the selection of chemicals for bioassay.

An extensive program of analyses and bioassays of tobacco smoke and its constituents aimed at the development of a less hazardous cigarette.

Major programs have been set up for the development of biological models for tumor pathogenesis closely correlated to the main types of cancers in man. Lung cancer and colon cancer programs are well established; pancreas cancer, endocrine cancers and childhood cancers programs are being developed. Their emphasis is on the use of animal models and human material to identify pathogenetic steps susceptible of inhibition or prevention.

In vitro models for neoplastic transformation by chemicals have been developed and are being used to establish their value both as screening techniques and as tools for identifying key steps in carcinogen activation and interaction, susceptible to inhibition. The applicability of these techniques to transformation tests of human cells is currently investigated.

Major advances have been made in the documentation of the conditions of formation and activation of several classes of carcinogens (nitrosamines, hydrazines, metals, etc.). Pharmacologic inhibition of the formation of carcinogens has been obtained by blocking nitrosation of amines.

Major metabolic steps in the enzymatic activation of several classes of carcinogens have been identified. Their reproducibility in human tissues has already been demonstrated in some cases. Micromethods, applicable to determinations in small samples of human cells, have been developed for measuring carcinogen activation levels. Inhibitors of carcinogen activation have been identified.

Important progress has been made in the application of immunologic methods to the study and control of carcinogenesis and tumor growth in animals and in man. These methods have also contributed valuable knowledge applicable to another program area, i.e. cancer immunotherapy in man.

A new program has been established to provide resources in somatic cell genetics as tools for the identification of major preventable steps in carcinogenesis.

Extensive consultation and documentation has been provided to DHEW, various governmental and other agencies and to Congress, on many problems of evaluation of environmental carcinogenic hazards and their prevention.

Major accomplishments in the Carcinogenesis Program in Fiscal Year 1972 are described in the reports from the Office of the Associate Scientific Director for Carcinogenesis, branches, and segments in the following sections of this report. Highlights of current achievements are described in the following paragraphs.

1. *PROGRAM DEVELOPMENT AND ORGANIZATION*

The total funding for Carcinogenesis has sharply increased in the current Fiscal Year.

This rapid expansion has been met with the organization of a management structure articulated in eight segments: *Bioassays* and *Tobacco Research* devoted to the identification of environmental carcinogenic agents and to the evaluation of "less hazardous" technological alternatives; *Biological Models, Lung Cancer,* and *Colon Cancer* devoted to the development of experimental models for studying the pathogenesis of the most important types of cancer seen in man, correlating human and animal observations with the objective of determining preventive measures applicable to specific cancer types in man; *Biology and Immunology* and *Chemistry and Molecular Carcinogenesis* devoted to the application of methods in cell biology, immunology, chemistry, and molecular biology to the problems of neoplastic transformation by chemical and physical agents, *in vivo* and *in vitro* to the identification of steps in the processes of carcinogenesis susceptible of inhibition or prevention and to the immunologic control or prevention of tumor formation; *Information and Resources* devoted to providing information and data analysis systems as well as biological and chemical resources needed for the entire program.

Each segment operates with a segment director for scientific programming, a segment manager, a staff of project officers, and an advisory group of outside scientists.

2. *BIOASSAYS FOR CARCINOGENIC ACTIVITY*

Carcinogenesis bioassays (resources) for feeding tests in mice and rats under a standardized procedure have been expanded to a level of 148 compounds in 80,000 animals in nine laboratories. The chemicals under test include 37 pesticides, 31 drugs, 26 dyestuff intermediates and aromatic amines, 18 solvents, 24 chemical intermediates, and 6 chlorodibenzodioxines. Ten other contracts use other specialized test systems for special groups of compounds.

Major chemical compounds that have been found carcinogenic under the conditions of these tests include several drugs (mostly cancer chemotherapeutic agents), pesticides, and industrial chemicals. Of importance in the high carcinogenic activity of substituted hydrazines (including the major constituent of rocket fuel), of several metal derivatives (particularly titanium compounds) and of almost all tested N-nitroso compounds. The results of these tests are being analyzed for final evaluation.

3. *TOBACCO RESEARCH*

The extensive program for the development of a less hazardous cigarette is expanding to include: selection, production and machine-smoking of some 40 types of cigarettes; collection of condensates of their smoke, chemical analysis and bioassay by various biological test systems; evaluation of pathologic effects and of inhibitory mechanisms.

4. *BIOLOGICAL MODELS*

New special programs aimed at developing specific experimental models for the major types of cancer seen in man, in order to identify pathogenetic steps

susceptible of prevention or inhibition, have been started in Fiscal Year 1972 to include: cancer of the pancreas, endocrine-related cancers (prostate and breast), kidney and bladder, tumors of childhood.

The synergism between diethylstilbesterol and X-irradiation in inducing breast cancer in rats has been confirmed even at lower doses of X-rays then previously used.

The common fungal toxin, aflatoxin, has been found highly carcinogenic when administered to newborn mice (a species previously thought to be resistant to this agent).

Transplantation studies in syngeneic hosts of chemically induced mouse liver tumors have shown the malignant nature of these tumors (often questioned in the evaluation of bioassay results). Several hepatocarcinogens were found to induce early production of alpha-feto-protein in rats, a useful marker which is common to animals and man.

A major advance has been made in obtaining *in vitro* transformation of cultures of liver cells.

Major metabolic steps in the activation of several carcinogens have been identified.

5. *LUNG CANCER*

Methods have been developed that permit us to study morphologic and biochemical events directly on the respiratory epithelium, using *in vivo* or *in vitro* exposure techniques, in animals and in human tissues. Specific early ultrastructural and biochemical changes induced by carcinogens have been identified. The role of anticarcinogenic substances is investigated. A major pathway of action of retinol in mucus synthesis has been identified as the control of transglycosylation reactions: its role in the control of respiratory cancer induction is actively studied.

Methods have been developed for isolating and characterizing the glycoproteins of lung and tracheal tissue. During vitamin A deficiency, there is almost complete disappearance, from the trachea, of a glycopeptide that is normally found in this tissue. This glycopeptide is virtually the only one containing the sugar, fucose.

The carcinogens, benzo[*a*]pyrene and diethylnitrosamine, have been found to produce similar preneoplastic, metaplastic lesions in both human and hamster tissues under similar types of exposure *in vitro* in organ culture.

Radioautographic studies have shown that pre-exposure of intact animals to benzpyrene greatly increases the subsequent binding of benzpyrene to cellular structures in tracheobronchial epithelium.

A method has been developed for obtaining exfoliative cytology from the respiratory tract of rats and hamsters and to follow the cytologic changes occuring during induction and early development of lung cancer. Cytological

specimens obtained from carcinogen-treated hamsters show a striking similarity to the sputum cytology obtained from humans during development of bronchogenic carcinoma.

Respiratory infection has been found to markedly enhance the incidence of lung tumors in rats exposed to the carcinogen N-nitrosoheptamethyleneimine, as well as impairing the ability of the lung to clear itself of particulate material.

Bis-(chloromethyl)ether, a widely used industrial alkylating agent, and one to which humans have been exposed in chemical plants, has been demonstrated to be an extremely potent respiratory carcinogen at exceedingly low inhalation doses in the rat. As a result of these studies, the precautions in the industrial use of this hazardous material have been greatly increased.

6. *COLON CANCER*

A new program has been established in close collaboration with the National Task Force on Colon Cancer. Its focus is on the definition of the correlation of colon cancer morbidity in migrant populations and the pathology of these cancers with environmental dietary factors. The role of bacterial flora in the metabolism of intestinal carcinogens and in the susceptibility of population groups is being studied.

7. *BIOLOGY AND IMMUNOLOGY*

The methods for *in vitro* transformation of cultured cells by chemical carcinogens are being defined and prepared for application as a screening procedure for carcinogenesis testing. The major finding of a rapid *in vitro* transformation of embryo cells exposed to carcinogens *in utero* by treatment of the pregnant mother animal has been extended to include several classes of chemical carcinogens: this methodology appears to be an excellent screening procedure for testing suspected human carcinogens in a short-term test.

Important progress was made in the use of immunologic methods for the study and control of carcinogenesis and tumor growth.

The need for live BCG in antitumor immunization has been circumvented providing a safer method for human application of this powerful new tool.

Identification of tumor-specific antibodies in sera of rabbits injected with chemically induced guinea pig tumors has been made possible by the development of a sensitive micro method. Tumor-specific antibodies were also demonstrated in guinea pigs against syngeneic tumor cells, an important first observation.

Immunologic recognition of previous exposure to carcinogens (acting as allergens) has been demonstrated in guinea pigs: these methods are being developed as a tool to identify human exposures to environmental carcinogens.

8. *CHEMISTRY AND MOLECULAR CARCINOGENESIS*

A new program on somatic cell genetics has been established to provide defined biologic materials (mutated cell lines with known biochemical markers) for the identification of major preventable steps in carcinogenesis. A major finding has been the temperature dependence of transformation induced by chemicals in hamster cells suggestive of a single gene mutation responsible for the maintenance of the transformed state.

Micromethods have been developed for the determination of carcinogen-activating microsomal enzymes, that can be determined in small biological samples including human cells, thus providing a tool for measuring carcinogen activation in man (e.g. screening of populations).

Over 50 chemicals are being tested for enzyme induction or inhibition in this context. Highly sensitive gel electrophoretic techniques are being further refined, showing, for the analysis of nucleic acids, that different base compositions can be recognized by electrophoretic mobility.

Major advances have been obtained in the documentation of the formation of carcinogenic nitrosamines from nitrites and secondary amines, an important source exposure to powerful carcinogens from hitherto unsuspected environmental sources.

Pharmacologic inhibition by ascorbate of the nitrosation of secondary amines by nitrites in the digestive tract, with consequent inhibition of their carcinogenic effect has been demonstrated and a simple and effective biological test has been developed to measure the pharmacologic block of this important reaction leading to the natural formation of carcinogens in the alimentary tract.

9. *INFORMATION AND RESOURCES*

A large scale feasibility study of an information system for ranking environmental chemicals for priority for carcinogenesis testing is nearing completion of its first phase. Major environmental exposure sources have been analyzed from the point of view of production, exposure, and suspected chemical activity.

A computerized biological data system has been put in operation for the collection and analysis of all bioassay data and results.

Other information and technical resources have been developed, including a procurement service for standard samples of purified carcinogens and analogs.

Evaluation of environmental cancer hazards has continued to require extensive documentation for use by various Government agencies.

Organization of conferences and workshops have been accomplished with the participation of members of the Carcinogenesis' staff. They include the following:

Lung Cancer Segment's Contractors and Advisors Conference held in Bethesda, Maryland, on November 3-5, 1971

Workshop on "Criteria for the Evaluation of Carcinogenic Hazards of Drugs" sponsored by the National Cancer Institute and the Food and Drug Administration held in Rockville, Maryland, on December 6-7, 1971, and April 6, 1972

Conference on "Virus Tumorigenesis and Immunogenesis" sponsored by the National Cancer Institute, the Eastern Pennsylvania Branch of the American Society for Microbiology, and the Department of Microbiology, College of Sciences, Pennsylvania State University held in University Park, Pennsylvania, on April 6-8, 1972

Conference on "Immunology of Carcinogenesis" sponsored by the National Cancer Institute and the Atomic Energy Commission (Oak Ridge National Laboratory) to be held in Gatlinburg, Tennessee, on May 8-11, 1972

International Chalone Conference--First Symposium--sponsored by the National Cancer Institute and the Office of Naval Research to be held in Augusta, Michigan, on June 5-7, 1972

Program Committee for Conference on "Host-Environment Interactions in the Etiology of Cancer in Man--Implementation in Research" sponsored by the Fogarty International Center, NIH, the International Agency for Research on Cancer, and the League for the Fight Against Cancer of the Croatian Republic of Yugoslavia to be held in Primosten, Yugoslavia, on August 27-September 2, 1972

World Symposium on "Model Studies on Chemical Carcinogenesis" sponsored by the National Cancer Institute and the Atomic Energy Commission to be held at Johns Hopkins Medical Institutions, Baltimore, Maryland, on October 31 - November 3, 1972

A general conference is being organized with participation of all advisors and contractors in the Carcinogenesis Program to be held in the Fall of 1972.

F. *FISCAL SUMMARY*

The following charts and tables report the fiscal history and the present distribution of funds in the Carcinogenesis Program, including Direct Operations and Contracts.

CARCINOGENESIS PROGRAM - FISCAL HISTORY

Description	June 1968	June 1969	June 1970	June 1971	June 1972 Current	June 1972 Projected
Personnel[1]	109	102	106	126	147	162
(Full-Time Permanent)	(107)	(98)	(96)	(110)	(129)	(131)
(Other Full Time)	(1)	(1)	(8)	(9)	(15)	(16)
(Part Time)	(1)	(3)	(2)	(7)	(13)	(15)
Space (In Sq. Ft.)	15,885	26,829[2]	26,112[3]	28,337	31,041[4]	
Level of Expenditure of In-House Operation	$1,636,000	$1,802,600	$1,997,000	$2,340,000	$3,460,000[5]	
Number of Contracts	33	40	51	69	91	129
Level of Expenditure of Contracts	$5,602,000	$8,466,600	$7,080,000	$10,598,000	$22,167,000[5]	

[1] Includes all personnel actually assigned to the Carcinogenesis Program.

[2] In November 1968, offices of the OASDC, located in the Wiscon Bg., and offices and laboratories of the Biology Branch, located in the Auburn Bg., moved to Building 37. In August 1969, offices and laboratories of the Chemistry Branch, located in the Auburn Bg., moved to Building 37.

[3] Experimental Pathology Branch and Units within the OASDC were established in January 1970.

[4] This amount includes space acquired from the Registry of Experimental Cancers which was transferred from the OD, NCI, and the space acquired in the Eye Research Foundation.

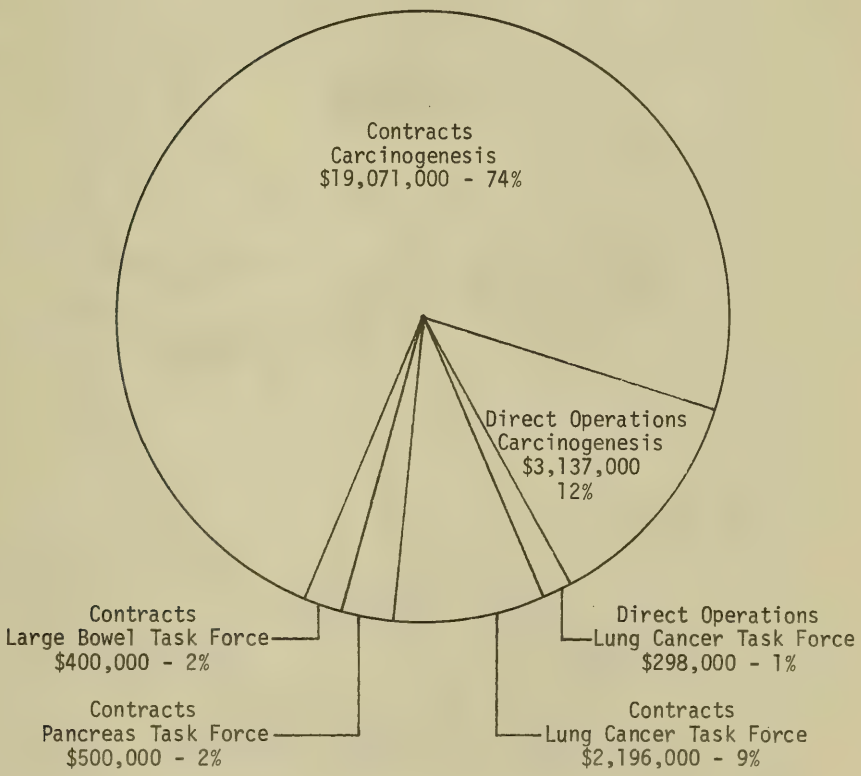
[5] Anticipated obligational level.

CARCINOGENESIS PROGRAM

ETIOLOGY AREA, NCI

Funding Obligations[1] - Direct Operations and Contracts

Fiscal Year 1972



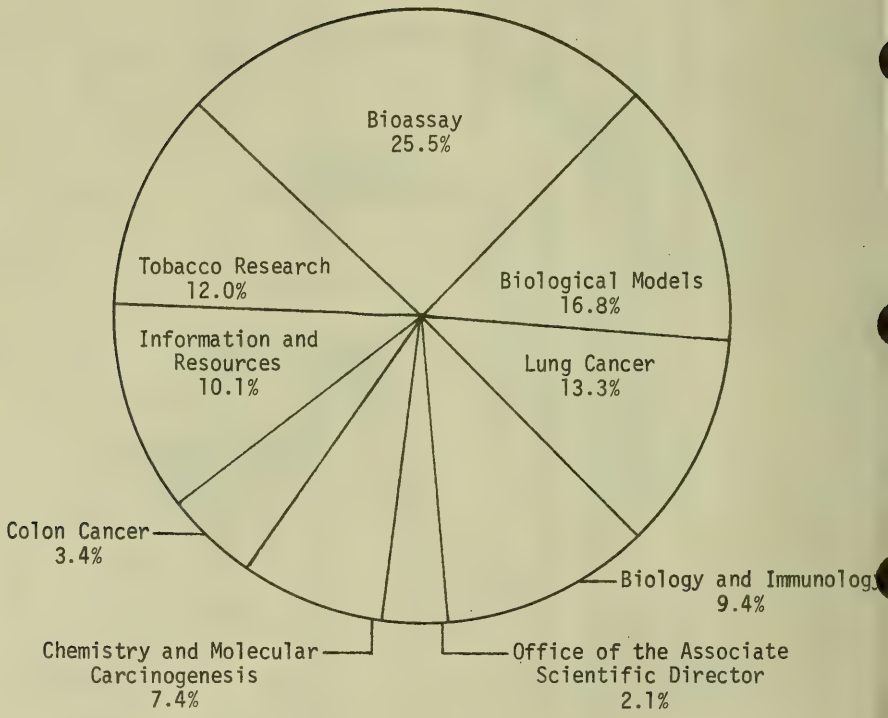
Total Contracts:	$22,167,000
Total Direct Operations:	3,435,000
TOTAL FUNDING:	$25,602,000

[1]Estimated FY 1972 Obligations, as of March 31, 1972

CARCINOGENESIS PROGRAM
ETIOLOGY AREA, NCI

Distribution of Contract Funds[1]

Fiscal Year 1972

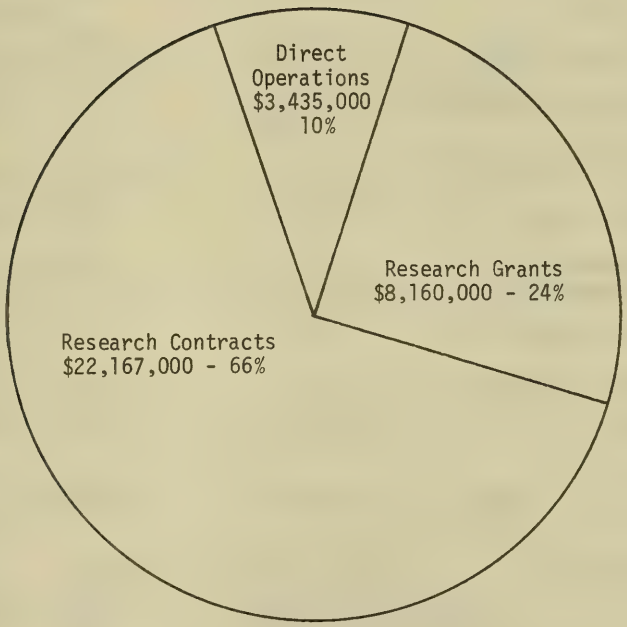


Total Contract Funds: $22,167,000

[1]Estimated FY 1972 Obligations, as of March 31, 1972

MAJOR SUPPORT MECHANISMS
FOR CARCINOGENESIS PROGRAMS
IN THE NATIONAL CANCER INSTITUTE

Fiscal Year 1972[1]



Total Funding for Carcinogenesis
Programs, NCI, FY 1972: $33,762,000

[1]Estimated FY 1972 Obligations, as of March 31, 1972

TABLE I

SUMMARY OF CONTRACT ACTIVITIES IN
THE CARCINOGENESIS PROGRAM

(dollars in thousands)

SEGMENT		NUMBER OF CONTRACTS	TOTAL AMOUNT	[1]PERCENT
I.	Bioassay	23	5,655	26
II.	Biological Models	25	3,720	17
III.	Biology & Immunology	20	2,077	9
IV.	Chemistry & Molecular Carcinogenesis	16	1,532	7
V.	Colon Cancer	5	746	4
VI.	Information & Resources	23	2,242	10
VII.	Lung Cancer	14	2,993	13
VIII.	Tobacco Research	9	2,667	12
IX.	OASD	2	467	2
	Total	[2]137	22,099	100

Allocations:	Carcinogenesis Contracts	19,071
	Lung Cancer Task Force	2,196
	Pancreas Task Force	500
	Large Bowel Task Force	400
	Total	22,167

[1]Total Estimated Through June 31, 1972.

[2]Several Contracts are listed more than one time.

TABLE II

CONTRACTS BY ACTIVITY IN
THE CARCINOGENESIS PROGRAM

(dollars in thousands)

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
I. Bioassay		$5,655
Bionetics Research Labs. NIH-NCI-E-69-2085	Determination of the Carcinogenicity of Chemicals Present in Man's Environment	$ 30
Bionetics Research Labs. NIH-NCI-E-71-2146	Carcinogenesis Bioassay of Environmental Chemicals	325
Bio-Research Consultants NIH-NCI-E-68-1311	Determination of the Carcinogenicity of Chemicals Present in Man's Environment	86
California, Univ. NIH-NCI-E-70-2206	Pulmonary Tumors in Mice for Carcinogenic and Co-carcinogenic Bioassay	148
Cincinnati, Univ. NIH-NCI-E-64-45	Determination of the Carcinogenic and Co-carcinogenic Properties of Industrial Chemicals	75
Columbia, Univ. NIH-NCI-67-731	Determination of the Carcinogenicity of Fresh and Mildly Oxidized Vegetable and Animal Fats	13
Gulf South Res. Inst. NIH-NCI-70-2210	Carcinogenesis Bioassay of Pesticides and Other Environmental Chemicals	500

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
Howard Univ. NIH-NCI-E-71-2167	Chemical & Biological Investigations of Potential Carcinogens from Plants	80
IIT Res. Inst. NIH-NCI-E-71-2338	Carcinogenesis Bioassay of Chlorinated Dibenzodioxans and Related Chemicals	320
Mason Res. Inst. NIH-NCI-71-2144	Carcinogenesis Bioassay of Environmental Chemicals	327
Massachusetts Inst. of Tech. NIH-NCI-E-62-468	Toxicity and Carcinogenesis Associated with Fungal Growth of Foodstuffs	174
Nebraska, Univ. NIH-NCI-E-68-959	Carcinogenesis Research and Bioassays	980
Papanicolaou Cancer Research Inst. NIH-NCI-E-64-80	Experiments Relevant to Studies in Chemical Carcinogenesis	49
Southern Res. Inst. NIH-NCI-E-68-998	Carcinogenicity of Chemotherapeutic Agents	292
TRW-Hazleton Labs. NIH-NCI-E-70-2209	Carcinogenesis Bioassay of Pesticides and Other Environmental Chemicals	445
TRW-Hazleton Labs. NIH-NCI-E-71-2145	Carcinogenesis Bioassay of Environmental Chemicals	433
Temple Univ. NIH-NCI-E-65-1029	Search for Carcinogens among Mycotoxins	87
Mass. Inst. of Tech. NIH-NCI-E-72-2076	Study of Interactions between Diet and Chemical Carcinogenesis in the Liver	62

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
To Be Awarded	Bioassay of Environmental Chemicals	258
To Be Awarded	Bioassay of Environmental Chemicals	399
To Be Awarded	Bioassay of Environmental Chemicals	136
To Be Awarded	Bioassay of Environmental Chemicals	283
To Be Awarded	Bioassay of Environmental Chemicals	153
II. Biological Models		$3,720
Alton Ochsner Medical Fdn. NIH-NCI-E-71-2131	Carcinogenesis by Radiation Plus Estrogen	80
Chicago, Univ. NIH-NCI-E-69-2087	Definition of Sensitivity of Carcinogenesis Bioassay Systems	320
Microbiological Asso. NIH-NCI-E-70-2199	Laboratory Service for Support in Carcinogenesis Bioassay and Related Activities	1,067
Nebraska, Univ. NIH-NCI-E-68-959	Carcinogenesis Research and Bioassays	210
San Francisco, Univ. NIH-NCI-E-64-886	Studies of Carcinogenicity of Metallo-Organic Compounds	104
Stanford Res. Inst. NIH-NCI-E-71-2166	Combined Effects of Chemical Carcinogens and Other Chemicals	304

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
Temple Univ. NIH-NCI-E-64-504	Research on the Biochemical and Morphological Components of Hepatic Carcinogenesis	35
Tennessee, Univ. NIH-NCI-E-69-2077	Carcinogenic Studies of Polyurethanes	68
Michigan, Univ. NIH-NCI-E-72-3216	Isolation and Purification of Epidermal Chalone	88
Bionetics Res. Labs. NIH-NCI-E-72-2063	Resource for Preparation and Examination of Experimental Biological Material for Histological and Ultrastructural Study	148
Nebraska, Univ. NIH-NCI-E-72-3212	Effects of Carcinogens on Organ Cultures (Mammary Glands)	43
Maryland, Univ. NIH-NCI-E-72-3206	Histogenesis of Renal Carcinomas	82
To Be Awarded	Organ Culture Development of the Pancreas	80
To Be Awarded	Enhanced Delivery of Chemical Carcinogens to the Pancreas	75
To Be Awarded	Development of Cell Cultures from the Pancreas	80
To Be Awarded	Development of Cell Cultures from the Pancreas	23
To Be Awarded	Development of Cell Cultures from the Pancreas	58

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
To Be Awarded	Comparative Endocrinology of the Prostate	100
To Be Awarded	Enhanced Delivery of Chemical Carcinogens to the Pancreas	111
To Be Awarded	Enhanced Delivery of Chemical Carcinogens to the Pancreas	145
To Be Awarded	Induction of Pancreatic Carcinoma in Guinea Pigs	43
To Be Awarded	Induction of Pancreatic Carcinoma in Guinea Pigs	86
Cor Bel Labs.	Animal Holding Facilities	100
To Be Awarded	Preparation of Cell Strains from Human and Animal Prostate	100
To Be Awarded	Induction of Adenocarcinoma of the Prostate	170
III. Biology & Immunology		$2,077
AEC NCI-FS-(64)-13	Research on Co-carcino genesis	154
Becton, Dickinson Res. Center. NIH-NCI-E-71-2168	Carcinogens as Allergens: Detection of Exposure to Carcinogens by Cell-Mediated Immunologic Reactions to the Carcinogens	222
Biolabs, Inc. NIH-NCI-E-71-2164	In Vitro Study of the Nature of Interaction between Chemical and Viral Carcinogens	191

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
Children's Hosp. Med. Center NIH-NCI-E-71-2278	Effects of Carcinogens on The In Vitro Synthesis of Complement Components	44
Cordis Corp. NIH-NCI-E-69-2076	Effects of Carcinogens on the Complement Non-Specific Immune System	0
Mallory Inst. of Path. NIH-NCI-E-71-2276	Detection of Carcinoembryonic Antigens in Humans	121
Meloy Inc. NIH-NCI-E-70-2201	Definition of Standard Experimental Conditions for a Reproducible In Vitro Carcinogenesis Bioassay	100
Nebraska, Univ. NIH-NCI-E-70-2203	Definition of Standard Experimental Conditions for a Reproducible In Vitro Carcinogenesis Bioassay	100
Utah, Univ. NIH-NCI-E-71-2272	Carcinogenesis Bioassay Resource for Determining the effect of Chronic Immunosuppression on Physical and Chemical Carcinogenesis	187
Scripps Clinic & Research Fdn. NIH-NCI-E-72-2046	Isolation & Chemical Characterization of Soluble Human Tumor (CEA) Specific Antigens	66
Trudeau Inst. NIH-NCI-E-72-3221	Tumor Inhibition by Mycobacteria: Standardization of Mycobacterial Preparations	156
Brandeis Univ.	Production and Detection of Antibodies to Chemical Carcinogens and Other Small Molecules	98

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
The Johns Hopkins Univ.	World Symposium on "Model Studies on Chemical Carcinogenesis"	20
To Be Awarded	Significance and Relationship of Fetoglobulins to the Induction of Hepatomas by Chemical Carcinogenesis	136
Ohio State Res. Fdn. NIH-NCI-E-72-2047	In Vitro Studv of the Nature of Interaction between Chemical and Viral Carcinogens	75
Texas, Univ. NIH-NCI-E-72-3210	Development of In Vitro Methods for the Detection of Cell-Mediated Immunologic Reactivity to Chemical Carcinogens	85
Case Western Res. NIH-NCI-E-72-3220	Specific Immunological Unresponsiveness to Chemical Carcinogens & Its Influence on Tumorigenesis	75
To Be Awarded	Induction of Malignant Melanomas in Guinea Pigs	100
Penn. State Univ. NIH-NCI-E-72-C-491	Conduct a Conference on Tumorigenesis and Immunogenesis	10
Univ. of Ill. Med. Ctr. NIH-NCI-E-72-3205	Research on the Transfer of Tumor Immunity Factor	137

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
IV. Chemistry & Molecular Carcinogenesis		$1,532
AEC NCI-FS-(64)-13	Research on Co-carcinogenesis	355
AEC NCI-FS-(64)-58	Investigation of the Molecular Processes Involved in the Carcinogenic Action of Polycyclic Aromatic Hydrocarbons	150
Home for the Jewish Aged. NIH-NCI-E-71-2269	Study of Serum Haptoglobin Types in Patients with Carcinoma of the Pancreas	35
The Johns Hopkins Univ. NIH-NCI-E-71-2169	Regulation of the Heme Moiety of P450 in Relationship to Carcinogen Metabolizing Activity	48
New York Univ. NIH-NCI-E-71-2020	Alkylating Agents As Carcinogens	120
New York Univ. NIH-NCI-E-71-2183	The Isolation, Propagation and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions	103

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
Texas, Univ. NIH-NCI-E-71-2268	Study of Serum Haptoglobin Types in Patients with Carcinoma of the Pancreas	20
Weizmann Inst. NIH-NCI-E-70-2217	Study of the Role of Enzyme Induction and Chemical Carcinogenesis	81
Illinois, Univ.	Temperature Sensitive Mutants in In Vitro Carcinogenesis	111
Ontario Cancer Inst. NIH-NCI-E-72-2051	The Isolation, Propagation, and Storage of Vertebrate Cells with Mutations in Specific Biochemical Functions	129
Polysciences, Inc.	Optimizing Electrophoretic Separation of Proteins with New Hydrogels	60
To Be Awarded	Nucleotide Sequence of Human Hemogloin-mRNA	100
To Be Awarded	Isolation and Characterization of Non-Histone Proteins Binding DNA	100
To Be Awarded	Isolation of Human Prolactin	120
To Be Awarded	Development of Mutant Vertebrate Cells	0
To Be Awarded	Synthesis of Polycyclic Hydrocarbon Metabolites	0

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
V. Colon Cancer		$ 746
American Health Fdn. NIH-NCI-E-71-2310	Experimental Large Bowel Carcinogenesis	350
Oxford Record Linkage System NIH-NCI-E-72-3215	Study of the Relationship in Individuals between Colon Cancer and Epidemiologically Linked Diseases	11
Cornell Univ. Med. College. NIH-NCI-E-72-2041	Analysis of Regulatory Control of Cell Proliferation in Normal, Premalignant and Malignant Colonic Tissues in Familial Polyposis	115
To Be Awarded	Epidemiology of Large Bowel Cancer Among U. S. Blacks and Spanish-American Migrants	70
To Be Awarded	Preparation of bile salt derivatives to study their role in the pathogenesis of large bowel cancer	200
VI. Information & Resources		$2,242
Ash-Stevens, Inc. NIH-NCI-E-66-929	Synthesis of Purine and Pyrimidine Nucleotides	150
Battelle-Columbus NIH-NCI-E-69-2251	Production of Animals for Support of Contracts 70-2210 (Gulf South Res. Inst.) and 70-2209 (Hazleton Lab.) under Supplement to Chemotherapy Contract 69-2251	96

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
Charles River Breeding Labs. NIH-NCI-E-72-2004	Production of Animals for Support of Contracts 70-2210 (Gulf South Res. Inst.) and 70-2209 (Hazleton Lab.) under Supplement to Chemotherapy Contract 66-87	12
IIT Res. Inst. NIH-NCI-E-70-2245	Preparation and Characterization of Particulate Materials for Studies in Respiratory Carcinogenesis	100
John I. Thompson NIH-NCI-E-69-2086	Literature Search and Retrieval of Specific Data Relating to Chronic Tests of Chemicals in Experimental Animals	72
John I. Thompson NIH-NCI-E-71-2266	Literature Search Retrieval and Compilation of Specific Data Relating to Chronic Tests in Experimental Animals	0
Mass. General Hospital NIH-NCI-E-71-2128	Atlas on Comparative Morphology & Classification of Spontaneous Neoplasms in Dog, Cat and Man	60
Massachusetts Inst. of Tech. NIH-NCI-E-70-2180	Environmental Occurrence of N-nitroso Compounds	75
Medizinische Hochschule NIH-NCI-E-71-2148	Development of a Lung Tumor Model with Large Wild Hamsters (Cricetus cricetus)	34
Miami, Univ. NIH-NCI-E-71-2274	Search for Possible Plant Causes of Esophageal Cancer	58

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
Minnesota, Univ. NIH-NCI-E-70-2058	Levels of Polycyclic Hydrocarbons in an Urban Atmosphere Based on the Particulate Size	1
Southwest Res. NIH-NCI-E-65-1000	Evaluation of Free Radicals and Alkylating Agents in Tobacco Smoke	22
Southwest Res. NIH-NCI-E-72-2065	Evaluation of Free Radicals and Alkylating Agents in Tobacco Smoke	89
Stanford Res. Inst. NIH-NCI-E-71-2045	Selection and Ranking of Environmental Chemicals for Bioassay	714
National Academy of Sciences	Support of Activities of the USA National Committee on Int'l Council Societies of Pathology (ICSP)	125
Chemotherapy, NCI	Standards for the Carcinogenesis Testing Program	145
To Be Awarded	Information Systems Microfilm Services to CBDS	140
Midwest Research Inst.	Analytical Chemical Resource	131
Franklin Inst. NIH-NCI-E-72-2040	Carcinogenesis Abstracts, Vol. X	93
Office of Naval Research	Chalone Conference	20
To Be Awarded	Contractor Interface with Program Management System	60

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
Data Systems	Provide WLN's Index for the Survey Books (PHS-149)	5
To Be Awarded	Stability of Benzo(a)Pyrene on Particulates Utilized in Respiratory Tract Studies	40
VII. Lung Cancer		$2,993
AEC NCI-FS-(64)-13	Research on Co-carcinogenesis	812
Battelle-North-West NIH-NCI-E-68-1372	Investigation of the Co-carcinogenicity of Specific Industrial Pollutants and Cigarette Smoke	181
California, Univ. NIH-NCI-E-70-2218	Correlation of Effects of Chemical Carcinogens in Human, Primate, and Hamster Epithelium In Vitro	35
Harvard School of Public Health NIH-NCI-E-71-2136	Factors Influencing Experimental Respiratory Carcinogenesis by Alpha Radiation and Chemical Carcinogens	80
IIT Res. Inst. NIH-NCI-E-69-2148	Study of the Role of Vehicles and Particulates in Respiratory Carcinogenesis Bioassays	297
Mass. Inst. of Tech. NIH-NCI-E-69-2083	Role of Vitamin A in the Control of Differentiation and Carcinogenesis in the Respiratory Tract	200

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
Medical College of Wisconsin NIH-NCI-E-69-2082	Autoradiographic Study of the Cellular Response of the Respiratory Tract in Chemical Carcinogenesis	111
Nebraska, Univ. NIH-NCI-E-68-959	Carcinogenesis Research and Bioassays	210
New York Univ. NIH-NCI-E-66-962	Studies in Pulmonary Carcinogenesis	306
Ohio St. Res. Fdn. NIH-NCI-E-69-2144	Study of the Role of Vehicles and Particulates in Respiratory Carcinogenesis Bioassays	118
Southern Res. Inst. NIH-NCI-E-72-2064	Organ Culture and Tissue Culture Bioassay of Vitamin A Analogs	165
St. Mary's Hospital	Morphogenesis of Human Lung Cancer	143
To Be Awarded	Characterization of Glycoproteins in Adenocarcinoma of Lung	100
To Be Awarded	Susceptibility States and Modulating Factors in Respiratory Carcinogenesis	235
VIII. Tobacco Research		$2,667
American Health Fdn. NIH-NCI-E-70-2087	Evaluation of Carcinogenic Agents in Cigarette Smoke. Biological and Chemical Assays and Epidemiological Studies	482

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
Arthur D. Little NIH-NCI-E-69-2147	Bioassay of the Cytotoxicity of Cigarette Smoke and its Effects on Ciliary Function	122
AEC NCI-FS-(64)-40-117-67	Collection, Separation, and Elucidation of the Components of Cigarette Smoke	813
Meloy Inc. NIH-NCI-E-69-2084	Preparation and Analysis of Cigarette Smoke Condensate Samples	358
New York Univ. NIH-NCI-E-64-938	Studies on Carcinogenesis Principles of Processed Tobacco and Tobacco Smoke	98
TRW-Hazleton Labs. NIH-NCI-E-71-2254	Chronic Carcinogenesis Bioassays by Intratracheal Instillation in Syrian Hamsters	60
TRW-Hazleton Labs. NIH-NCI-E-69-2145	Carcinogenicity Bioassays by Intragastric Intubation of Cigarette Smoke Condensates in Experimental Animals	114
TRW-Hazleton NIH-NCI-E-69-2149	Skin Carcinogenesis Bioassay of Cigarette Smoke Condensates in Mice	312
VA Hospital	Test Effects of High and Low Nicotine Cigarette on Male Beagle Dogs	308
IX. Office Of Associate Scientific Director		$ 467
AEC NCI-FS-(64)-13	Role of Nitrosamines in Cancer.	384

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT
Columbia Univ.	Develop Tissue Culture Transformation System for Aromatic Amine Carcinogens	83

II. *OFFICE AND BRANCH REPORTS*

SUMMARY REPORT

OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR FOR CARCINOGENESIS

July 1, 1971 through June 30, 1972

The direct operation of the Office of the Associate Scientific Director for Carcinogenesis includes the immediate Office of the Associate Scientific Director for Carcinogenesis (U. Saffiotti, Associate Scientific Director; J. A. Cooper, II, Acting deputy Associate Scientific Director; A. H. Heim, Coordinator of Collaborative Research), the Program and Data Analysis Unit (N. P. Page, Acting Head), the Lung Cancer Unit (M. B. Sporn, Head), and the Epidemiologic Pathology Unit (J. W. Berg, Head). An Analytical Chemistry Unit is now being established and the Registry of Experimental Cancers is being transferred from the OD, NCI.

The planning and coordination of the whole Carcinogenesis Area program has continued to take the major effort of this Office, most effectively assisted by the development of special monitoring systems in the Program and Data Analysis Unit. A new management structure for the Carcinogenesis Program has been established as described in Section I-C of the present report.

This Office has continued to serve as the focal point for the development of documentation on the assessment of environmental carcinogenesis hazards and on the principles for evaluation of environmental carcinogens, in collaboration with the Biometry Branch, Demography, Etiology, and with the Office of the Director, NCI. In this capacity this Office has participated in a number of review and policy meetings at the request of other government departments and agencies, including: The Council on Environmental Quality, the President's Science Advisory Committee, and the Office of Science and Technology, Executive Office of the President; Office of the Assistant Secretary for Health and Scientific Affairs, DHEW; Office of the Surgeon General, USPHS; the Office of the Commissioner and the Bureaus of Foods and of Drugs, FDA; Advisory Groups for National Center for Toxicological Research, FDA; the Drug Advisory Board, National Research Council--National Academy of Sciences; and several committees and ad hoc groups with FDA, EPA, USDA, and other agencies.

Testimonies were given by the Associate Scientific Director at the hearings of the Subcommittee on Intergovernmental Relations of the Committee on Government Operations, House of Representatives (Chairman: Rep. L. H. Fountain) on Regulation of Diethylstilbesterol and at the hearings of the Environmental Protection Agency on DDT.

The Office also participated in the activities of the Working Group on the Evaluation of Carcinogenic Risks of Chemicals to Man, International Agency for Research on Cancer, Lyon, France, and in the Program Committee for the 1972 Conference on Epidemiological Approaches to Carcinogens sponsored by

the Fogarty International Center, NIH, the International Agency for Research on Cancer, and the League for the Fight Against Cancer of the Croatian Republic of Yugoslavia. This Office also participated in the organization of several meetings as reported in Section I-F.

These and other related activities in the area of evaluation of environmental hazards have required a large share of the resources of this Office, including communication with congressional committees, other government agencies, the press, and the information offices at NCI, NIH, and DHEW. Major emphasis has been given in all these activities to the unique role of NCI in providing documentation of carcinogenesis bioassay studies and scientific criteria for evaluation of carcinogenic hazards for man.

The major effort of this Office has been devoted to the scientific planning, development, coordination, and implementation of a considerable expansion in resources and activities assigned to the Carcinogenesis Program as a result of the increased funding level for NCI in Fiscal Year 1972. Documentation of this effort is provided throughout this Report.

PROGRAM AND DATA ANALYSIS UNIT - This Unit provides the Associate Scientific Director with staff support in information and data processing, program planning and evaluation, management of animal and chemical resources, as well as expertise in radiation biology. Doctor J. A. Cooper had served as Unit Head until March 1971 when Dr. N. P. Page was appointed Acting Head. The staff of the Unit has cooperated with the Coordinator of Collaborative Research in the evolution of the contract management system now in use. In addition to their intramural activities, Dr. Cooper serves as Director of the Information and Resources Segments. Doctor Page served as Manager for the Lung Cancer Segment until February 1972; Dr. S. Siegel serves as Manager of the Information and Resources Segment. Members also serve as project officers on a number of contracts, particularly those dealing with chemical and information resources and radiation carcinogenesis. The following is a summary of the main areas of effort during the past year.

(1) *Information Storage and Retrieval Systems* - The Carcinogenesis Bioassay Data System (CBDS) designed by this Unit in collaboration with the NIH Division of Computer Research and Technology (DCRT), is used to collect, monitor, and store experimental data and results from bioassay operations. It is designed for complete or selective recall of bioassay data. The System is now in full operation with new bioassay experiments being entered as they are initiated. Data from 11 bioassay contractors are now on file with several other contractors preparing data for entry into the System. During the coming year the staff will expand the System to permit the analysis of data using those methods best suited to the interpretation of carcinogenesis data. In addition, the Unit maintains a Carcinogenesis Chemical Information System which lists data on materials presently under test or projected for study. The Unit previously supported the Carcinogenesis Contract Data System as a central source of administrative data on the contract program. This function has now been turned over the program management system developed by the staff of the Associate Scientific Director for Program, Etiology, NCI.

(2) *Information Resources* - The Unit serves as a focal point for the acquisition and publication of information pertaining to the Carcinogenesis

Program. The staff provides editorial advice and collaboration for the following publications produced under contracts:

(a) *Survey of Compounds Which Have Been Tested for Carcinogenic Activity* (PHS Publication No. 149) - This publication represents a standard reference data base in the field of chemical carcinogenesis bioassays (Contractor: John I. Thompson Co.).

(b) *Carcinogenesis Abstracts* - This is a monthly publication which provides the scientific community with current abstracts on all aspects of carcinogenesis research (Contractor: Franklin Research Institute). The volume of information deemed relevant has increased dramatically in the last fiscal year necessitating expansion of this information activity.

(c) Searches conducted on a variety of subjects utilizing data bases such as the Smithsonian Information Exchange, MEDLARS, and National Library of Medicine Toxicology Information Program. In addition, a pilot program for selective dissemination of information (SDI) has developed in collaboration with the U. S. Department of Agriculture to assist program scientists in maintaining currency in their specific areas of interest. The SDI approach to literature evaluation is able to make available to program scientists information which is not only timely but also highly pertinent to their research activities. This SDI system allows a degree of specificity in requesting information that no other system including MEDLARS can offer. Approximately ten program scientists are receiving information from this system.

(3) *Chemical and Animal Resources* - Capabilities are being developed under our contract program for providing adequate quantities of well-characterized carcinogenic chemicals and related compounds for use in program activities. The Unit recognized a need to develop a more extensive program to meet the animal requirements of the Carcinogenesis Program and is assisting the program directors in planning their animal needs as far in advance as possible, coordinating these needs with the animal supplies and providing information on the characteristics and availability of laboratory animals. With the recently enacted Animal Welfare Act, assurances of compliance by both contract and intramural programs are necessary. Doctor Page is currently chairman of an NCI ad hoc group appointed for that purpose.

(4) *Other Activities* - The Unit has assisted in the analysis of data as requested by program leaders in Carcinogenesis. An example of this activity was the in-depth analysis of bioassay results on nitrilotriacetic acid (NTA) undertaken at the request of the Surgeon General, PHS. The Carcinogenesis Program has a limited but significant program in radiation carcinogenesis on the interactions of radiation and chemicals and mechanism of action. Doctor Page acts as the coordinator and project officer for these studies.

LUNG CANCER UNIT - The intramural program of this Unit makes use of a broad spectrum of experimental techniques in a coordinated program of studies on the pathogenesis of lung cancer and its inhibition. Current studies include

investigations on the carcinogenic effects of different chemicals on the respiratory epithelium in the whole animal, the definition of the short-term effects of carcinogens on *in vitro* organ culture systems, investigations of the molecular mechanism of action of such carcinogens in cell-free systems, and the molecular mechanism of action of the anticarcinogenic substance, vitamin A. The intramural research program is closely coordinated with the collaborative program of the Lung Cancer Segment. Dr. M. B. Sporn, Unit Head, serves as Director of the Lung Cancer Segment, and the Unit's professional staff also provides project officers to its contracts.

The following is a summary of the investigations within the intramural program of the Lung Cancer Unit:

(1) *Animal Studies of Respiratory Carcinogenesis (Morphological and Biochemical Correlations)* - The effects of various dosage schedules for intratracheal administration of polycyclic hydrocarbons, nitrosamines and/or other material in induction of squamous cell carcinoma in respiratory epithelium of hamsters are being studied. The long-term animal studies are paralleled with studies of short-term effects. Some of the parameters being measured are incidence and type of tumors, ultrastructural effects of carcinogens on respiratory epithelium, and biochemical changes induced in respiratory epithelium in response to the administration of the carcinogen. The last problem is being investigated in particular detail with respect to changes in RNA synthesis and processing which may be induced by carcinogenic agents such as benzpyrene. Methods have been developed for separating respiratory epithelium from underlying connective tissue and cartilage, and for preparing metabolically active subcellular particles, such as nuclei, from the epithelial cells. Tracheal nuclei from animals treated with the carcinogen, benzo[*a*]pyrene, have been found to have abnormally high enzymatic activity for RNA synthesis. Tracheobronchial epithelial cells from animals treated with benzo[*a*]pyrene have been examined by electron microscopy. Many of these cells were found to have abnormal features similar to those found in hyperplastic epithelial cells described in the bronchi of smoking dogs and to those found in neoplastic cells of human bronchogenic carcinoma. Moreover, the squamous metaplasias caused by carcinogens can be distinguished, at the electron microscopic level, from the squamous metaplasia that occurs in a benign state, such as vitamin A deficiency.

(2) *Studies on the Isolated Tracheal Epithelium of the Hamster (Short-Term Organ Culture)* - Methods have been developed for short-term organ culture of tracheas, during which time the epithelium may be exposed to any of a variety of radioisotopes. The epithelium is then scraped away from the rest of the trachea, and studies on the metabolism of the radioactive label can then be performed. Before the trachea is placed in the short-term organ culture, the donor animal may be treated with any of a variety of carcinogenic or anticarcinogenic substances. This technique has been used particularly in conjunction with polyacrylamide-gel-electrophoretic and radioautographic studies of RNA metabolism, with the carcinogens studied being benzpyrene, nitrosomethylurea, and diethylnitrosamine. The anticarcinogenic substance has been vitamin A. Water soluble carcinogens, such as nitrosomethylurea and diethylnitrosamine, can be applied directly to the isolated trachea and can be shown to produce ultrastructural lesions in short-term studies. In

studies employing the above short-term organ culture methods, the following new findings have been made: (a) vitamin A has been found to be necessary for the proper formation of high molecular weight RNA, (b) prior treatment of animals with the carcinogen, benzo[$_a$]pyrene, has been found to markedly increase the subsequent binding of benzo[$_a$]pyrene to tracheal epithelial cells; (c) mucus cells were found to incorporate significant amounts of thymidine into their nuclei, and (d) the carcinogen, nitrosomethylurea, was found to produce cellular lesions which persist upon transplantation of the treated tracheas into the cheek pouch of normal hamsters.

(3) *Studies on the Cellular and Biochemical Mechanism of Action of Vitamin A* - Since vitamin A is a required substance for normal differentiation of respiratory epithelium and since it inhibits the effects of carcinogenic substances such as benzpyrene, both *in vivo* and *in vitro,* studies on the mechanism of action of this substance are being actively pursued. These studies include electron microscopic examination of respiratory epithelium, during both hypo- and hypervitaminosis A, as well as biochemical investigations on the role of vitamin A in controlling glycoprotein and mucus synthesis in normal and malignant epithelial cells. In these biochemical studies, the molecular involvement of vitamin A alcohol (retinol) in transglycosylation reactions in mucus secreting epithelia has been demonstrated. Retinol has been shown to form polar intermediates, containing sugar-phosphate esters, which function as carriers for hexose sugars in the biosynthesis of epithelial mucins and of surface glycoproteins.

(4) *Studies on the Control of Nuclear RNA Metabolism in Tracheal Epithelial Cells* - Since abnormal nuclear and nucleolar morphology are frequently used criteria for diagnosis of the malignant state in respiratory epithelium, studies on the factors controlling synthesis and processing of nuclear RNA are being conducted. These studies are currently focusing on the role of the nucleolus in 2'-o-methylation (sugar methylation) of RNA, and the effect of respiratory carcinogens on this process. Within the Lung Cancer Unit, new biochemical methodology, including specialized techniques of gel electrophoresis and chromatography of RNA, has just been developed, which will allow extensive new molecular studies on the role of RNA methylation in respiratory carcinogenesis. The chromatographic method that has been developed allows, for the first time, the separation of all four commonly occuring sugar methylated ribonucleosides from each other, as well as from all base-methylated or non-methylated ribonucleosides. Using this method, it has been shown, for the first time, that isolated mammalian cell nuclei are capable of sugar-methylation of all four RNA nucleosides. These studies are now in the process of being applied to an analysis of the effects of nitrosamine carcinogens on sugar methylation of RNA in liver and tracheobronchial nuclei.

EPIDEMIOLOGIC PATHOLOGY UNIT - This Unit represents the major link between the Carcinogenesis Program and the Demography Program in Etiology, and provides expertise and advice to the whole Carcinogenesis Area. Most of the efforts of the Unit's staff are now devoted to the work of the Colon Cancer Segment and to related contract programs. Doctor J. W. Berg, Head of the Unit, serves as Director of the Colon Cancer Segment, as member of two segment advisory groups, as well as project officer or assistant project officer on sixteen contracts.

Doctor S. Silverman has joined the staff with responsibility for the bacteriology program of the Segment and for the expected activities at Ft. Detrick. Doctor M. Howell has joined the staff to take charge of the epidemiologic studies of the segment and to assist in the analysis of the Unit's epidemiologi pathology data. This will be particularly important now that initial data are being received from the Third National Cancer Survey. Mr. H. Feldman returned to Harvard University after two years as the Unit's programmer: his accomplishments in his other role of mathematician are described below. Consultative work has continued particularly in regard to the migrant program, the Third National Cancer Survey, the Lung Cancer Program, and others.

Correlations between trace metals in water supplies and cancer mortality showed that nickel concentrations correlated with mouth and intestinal cancer death rates; arsenic concentrations with mortality from larynx cancer, eye cancer and myeloid leukemia; beryllium, which produces bone cancer in animals, was correlated with bone cancer mortality; lead, a renal carcinogen in animals and a hematopoietic poison was correlated with mortality from kidney cancer, leukemias, and lymphomas; cadmium concentrations were correlated with multiple cancer sites and with death rates from all cancers.

Doctor Lingeman's work on gastrointestinal neoplasms in man and animals has provided the solid foundation for our efforts to link experimental and human disease. She also examined mammary gland neoplasms of female cats. Most feline mammary gland neoplasms are ductal adenocarcinomas resembling human cancer more closely than do breast cancers of any other species.

In cancer pathology studies, a quantitative analysis of axillary nodes in breast cancer patients has yielded a quite unexpected finding: no matter how much or how little cancer was present in the axilla, the patient's prognosis was directly related to the amount of benign node tissue present in the apical nodes.

The extranodal lymphomas in the End Results Registries were analyzed and the important differences noted between these tumors, lymphomas arising in nodes, and cancer arising from the corresponding organ epithelium.

Starting with a simple model of radioimmunoassay, Mr. Feldman and his colleagues developed a comprehensive theory for predicting dose-response relationships for the case of several antigens (or ligands) and several antibodies (or binding proteins) with different pairwise affinity constants under a variety of assay conditions.

Serial No. NCI-4696
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Evaluation of Carcinogenic Hazards

Previous Serial Number: Same

Principal Investigator: Umberto Saffiotti, M.D.

Other Investigators: Collaboration from the Staff of the Carcinogenesis Area

Cooperating Units: Collaboration from the Staff of the Demography Area and Outside Advisors

Man Years:

Total:	0.3
Professional:	0.3
Other:	0.3

Project Description

Objectives:

To examine current knowledge in the field of carcinogenesis and related fields and to identify criteria for the evaluation of carcinogenic hazards by chemical and physical agents and for the prevention of their carcinogenic effects in man. To examine laboratory findings on the biological action of chemicals and to review data on exposures for man in order to formulate a scientific evaluation of their carcinogenic risk.

Methods Employed:

Scientific coordination of intramural and collaborative programs in the Carcinogenesis Program, Etiology, NCI. Convening expert advisory groups. Consultation with other government agencies and with members of the scientific community. Review of scientific information. Individual analysis of data collected in this field and particularly results obtained in carcinogenesis experiments.

Major Findings:

A review of scientific data on "The Role of Dose Response Studies in the Evaluation of Carcinogenic Hazards" is being prepared for publication.

A detailed analysis of carcinogenesis bioassay data on nitrilotriacetic acid (NTA)--a detergent additive proposed for extensive use--was prepared, including review of histological material and of experimental results. A report has been submitted to the Office of the Surgeon General, USPHS.

Numerous contributions have been made towards the establishment of national policies in the field of carcinogenesis hazards, through discussions and documentation provided to Congressional Committees, government agencies, and offices, and to scientific societies.

Significance to Biomedical Research and the Program of the Institute:

The national policies on environmental health and cancer prevention should be based on sound scientific grounds. The Carcinogenesis Program at NCI provides a central source of information and competence in this field.

Proposed Course of Project:

Continuation and expansion of these functions.

Honors and Awards

Recipient, DHEW Superior Service Honor Award, June 1971

Chairman, Carcinogenesis Advisory Panel, Etiology, NCI

Chairman, Carcinogenesis Contract Program Management Group, Etiology, NCI

Chairman, Carcinogenesis Intramural Program Group, Etiology, NCI

Member, Etiology Program Management Group, NCI

Member, Tobacco Working Group, Lung Cancer Task Force, NCI

Member, Working Group on the Evaluation of Carcinogenic Risk of Chemicals to Man, International Agency for Research on Cancer, World Health Organization

Consultant, Advisory Group on Drug-Related Carcinogenesis of the Food and Drug Administration

Participant, Ad Hoc Committee on NTA, Office of the Assistant Secretary for Health and Scientific Affairs, DHEW

Member, Program Committee for the 1972 Conference on Epidemiological Approaches to Carcinogenesis sponsored by the Fogarty International Center, NIH, the IARC, and the League for the Fight Against Cancer of the Croatian Republic of Yugoslavia to be held August 1972

Attendant, Panel on Chemicals and Health of the President's Science Advisory Committee, Office of Science and Technology, Executive Office of the President, February 1971

Speaker, Ninth Canadian Cancer Research Conference, sponsored by the National Cancer Institute of Canada, held in Honey Harbour, Ontario, June 1971

Participant, International Bladder Cancer Conference, sponsored by the International Union Against Cancer, held in Leeds, England, September 1971

Participant and Liaison for the National Cancer Institute, Workshops on Carcinogenesis organized by the Interdisciplinary Communications Program, Smithsonian Institution

Lecturer and Participant, Environmental Pathology Seminar Series sponsored by the Temple University School of Medicine, Department of Pathology, December 1971

Organizer and Participant, Joint FDA-NCI Conference on Criteria for Evaluation of Carcinogenic Hazards of Drugs, December 1971

Member, Planning Meeting on The Role of the Pathologist in the Design and Conduct of Toxicology Studies organized by the Universities Associated for Research and Education in Pathology, Inc. and the Life Sciences Research Office of the FASEB, January 1972

Participant, Ad Hoc Subcommittee on Clinical Relevance of Carcinogenicity Testing with Drugs of the Committee on Problems of Drug Safety, Drug Advisory Board, National Academy of Sciences, National Research Council, January 1971

Member, Ad Hoc Group Meeting at the New York Academy of Sciences to advise on initiation and development of a Society of Occupational Toxicology, February 1972

Speaker and Participant, Protocol Task Force for the National Center for Toxicological Research, Food and Drug Administration, April 1972

Speaker, Toxic Substances Group, Council on Environmental Quality, Office of Science and Technology, Executive Office of the President, April 1972

Chairman, Session on Carcinogenesis, at the 63rd Annual Meeting of the American Association for Cancer Research, Boston, Massachusetts, May 1972

Publications

Harris, C. C., Sporn, M. B., Kaufman, D. G., Smith, J. M., Baker, M. S., and Saffiotti, U.: Acute ultrastructural effects of benzo[*a*]pyrene and ferric oxide on the hamster tracheobronchial epithelium. *Cancer Res.* 31: 1977-1981, 1972.

Harris, C., Sporn, M., Kaufman, D., and Saffiotti, U.: Histogenesis of squamous metaplasia in the hamster respiratory epithelium caused by vitamin A deficiency or by benzo[*a*]pyrene-ferric oxide. *J. Nat. Cancer Inst.* 48: 743-747, 1972.

Saffiotti, U.: Food additives in relation to environmental cancer in man. In Clark, R. L., Cumley, R. W., McCay, J., and Copeland, M. M. (Eds.): *Oncology 1970: A. Environmental Causes; B. Epidemiology and Demography; C. Cancer Education* (Proceedings of the Tenth International Cancer Congress). Chicago, Illinois, Yearbook Medical Publishers, Inc., Vol. 5, 1972, pp. 238-240.

Saffiotti, U.: Mechanisms of cancer induction in relation to the problem of environmental cancer. In *Environment and Cancer* (24th Annual Symposium on Fundamental Cancer Research). Baltimore, Maryland, Williams and Wilkins (In Press).

Saffiotti, U.: The laboratory approach to the identification of environmental carcinogens. In *Canadian Cancer Conference* (Proceedings of the Ninth Canadian Cancer Research Conference). Toronto, Canada, University of Toronto Press (In Press).

Saffiotti, U., Montesano, R., Sellakumar, A. R., Cefis, F., and Kaufman, D. G.: Respiratory tract carcinogenesis in hamsters induced by different numbers of administrations of benzo[*a*]pyrene and ferric oxide. *Cancer Res.* (In Press).

Serial No. NCI-4398
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Epidemiologic Pathology Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Registry Based Pathology Studies of Cancer

Previous Serial Number: Same

Principal Investigator: John W. Berg, M.D.

Other Investigators: Margaret Howell, Andrew Huvos and Guy F. Robbins (Memorial Hospital for Cancer and Allied Diseases), Grant Stemmerman and Nubuki Susano (Connecticut Cancer Registry), and Coy Freeman and Stephen Baylor (Biometry Branch, Demography, NCI).

Cooperating Units: None

Man Years:

Total:	2.6
Professional:	1.9
Other:	0.7

Project Description

Objectives:

(1) To analyze pathology data now existing in tumor registries by study of epidemiology and survival correlations; (2) To utilize existing registry and related data in the solution of specific pathology problems such as the significance of specific histologic patterns and the behavior of rare tumors; (3) To discover what further pathology data are needed and could be used in epidemiological and end-result studies and which types of registry data are particularly needed to help solve problems in epidemiological pathology; (4) To plan for and to collect such data in a form suitable for modern types of computer-based statistical analysis; and (5) To undertake studies in quantitative cancer pathology and to base as many of these as possible on registry-defined data.

Methods Employed:

Computer surveys of large record files, record review of specific sets of cases, statistical analyses, and qualitative and quantitative microscopic studies of particular pathology material all are used when appropriate.

Major Findings:

(1) Studies on nomenclature and classification of tumors have been expanded. Collaboration with the National Office of Health Statistics continues in preparing recommendations for the forthcoming 9th Revision of the International Classification of Diseases. The Unit's unique contribution has been to furnish and interpret real data on tumor types and locations so that decisions will be based on facts rather than on unsubstantiated theorizing, as has been usually true in the past. The past year has seen some additional work on cancers but most effort has been in the relatively untouched area of nonmalignant tumors (many of which can be precursors of cancer). The 1968 Edition of the American Cancer Society's Manual of Tumor Nomenclature and Coding has been published and studies on the conversion of cancer registry data to this more powerful coding scheme are continuing.

(2) A quantitative analysis of axillary nodes in breast cancer patients has now been completed. Two quite unexpected findings emerged: (a) The size and tumor content of most of the axillary nodes have no relation to clinical evaluation. Correct judgments were made only when there were nodes at the apex of the axilla large enough to be felt. (b) These same apical nodes have a special role in host defense. No matter how much or how little cancer was present in the axilla, the patient's prognosis was directly related to the amount of benign node tissue present in the apical nodes. This work was done on case material from Memorial Hospital, working from pathologic specimens and analyses of the clinical variables previously carried out by the End Results Section, Biometry Branch, Demography, NCI.

(3) Epidemiological-pathological studies of specific types of cancer are continuing. In collaboration with the WHO Reference Center for Intestinal Tumors, bowel cancers were analyzed from this standpoint using histological material from the Migrant Studies, unpublished registry data from the Connecticut Cancer Registry, from the Memorial Hospital, and from Sendai, Japan, as well as published data. Histology was found to be related to age, sex, and location within the bowel, but not to geography. In a paper now in preparation, the physical dividing line between cancers that are epidemiologically rectal and epidemiologically colon is completely redefined.

(4) Lymphomas arise from sites other than lymph nodes with an unappreciated high frequency. In the End Results Section's data file, for instance, 24% of all lymphomas other than Hodgkins' Disease were considered of extra-nodel origin. The case material of the End Results Section was analyzed and the important differences noted between these tumors, lymphomas arising in nodes, and cancer arising from the corresponding organ epithelium. Each one of the organ-specific lymphomas has its own pattern of presentation and behavior and, for practical purposes, these are more important than the underlying theoretical similarities we noted. In particular, it was emphasized that many extra-nodal lymphomas were cured by local treatment, a fact often lost sight of.

(5) A similar study is now in progress on the different histologic varieties of pancreatic cancer. Most specific varients of the common adenocarcinoma are so uncommon that only from a data bank as large as the End Results Section

can case material be assembled that show the differences and importance of the specialized tumor types. Once the differences are documented, however, we can expect pathologists to be more specific in their diagnoses, and it may be that epidemiologically important differences may emerge.

Significance to Biomedical Research and the Program of the Institute:

While individual studies hopefully have intrinsic value, the prime aim of the project is to provide a broad factual framework for use in planning the programs of the Etiology Area. In particular, it aims for a more specific identification of the heterogenous assortment of diseases including "cancer" of the various anatomic sites and for development of more precise and rigorous techniques for quantitative description of human cancers.

Proposed Course of Project:

As studies now underway are completed, others will be implemented with consideration to the priorities within the Carcinogenesis Program and the Etiology Area and also with the capabilities and resources of the Unit's activities. In addition, we hope that we can promote greater utilization of our pathology data bank which is probably the largest in the world.

Honors and Awards

Member, Task Force on Lung Cancer, American Joint Committee on Cancer Staging and End Results Reporting

Member, Task Force to Review "Manual of Tumor Nomenclature"; Member, Clinical Investigation Advisory Committee; Member, Lung Cancer Pathology Advisory Committee; American Cancer Society

Consultant, Epidemiologic Pathology, Memorial Hospital for Cancer and Allied Diseases

Member, Working Party on Classification of Neoplasms of the Revision of the International Classification of Diseases, U.S. National Committee on Vital and Health Statistics; WHO Working Group on Chapter II: Neoplasms of the 9th Revision of the International Classification of Diseases

Member, Board of Editors, Gastroenterology Section, *Excerpta Medica*

Member, Planning Group, National Program for Colon Cancer, NCI

Member, Carcinogenesis Contract Program Management Group, Etiology, NCI

Director, Colon Cancer Segment, Carcinogenesis Program, Etiology, NCI

Member, Lung Cancer Segment and Biological Models Segment, Carcinogenesis Program, Etiology, NCI

Invited Speaker, APHA Symposium on the Epidemiology of Colon Cancer, October 1971

Invited Speaker, Second National Conference on Breast Cancer, May 1971

Invited Speaker, Johns Hopkins School of Public Health, October 1971

Invited Speaker, Symposium on Geographic Pathology, Latin American Society of Pathology, December 1971

Publications

Berg, J. W.: Morphological evidence for immune response to breast cancer. *Cancer* 28: 1453-1456, 1971.

Freeman, C., Berg, J. W., and Cutler, S. J.: Occurrence and prognosis of extranodal lymphomas. *Cancer* 29: 252-260, 1972.

Berg, J. W., Huvos, A. G., Axtell, L. M., and Robbins, G. F.: A new sign of favorable prognosis in breast cancer: Hyperplastic reactive lymph nodes in the apex of the axilla. *Ann. Surg.* (In Press).

Serial No. NCI-4397
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Epidemiologic Pathology Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Comparative Epidemiology of Malignant Neoplasms of Man and Animals

Previous Serial Number: Same

Principal Investigator: Carolyn H. Lingeman

Other Investigators: F. M. Garner, Armed Forces Institute of Pathology

Cooperating Units: Epidemiology and Biometry Branches, Demography; Experimental Pathology Branch, Carcinogenesis; Etiology

Man Years:
Total: 2.6
Professional: 1.9
Other: 0.7

Project Description

Objectives:

To evalute epidemiologic features of spontaneous malignant neoplasms of man and animals to attempt to discover factors involved in their etiologies.

Methods Employed:

In cooperation with institutions with human and animal tumor registries, we are investigating the epidemiologic and morphologic characteristics of prevalent neoplasms of man and animals: (1) hematopoietic neoplasms, (2) gastric adenocarcinomas, (3) intestinal adenocarcinomas, and (4) mammary gland carcinomas.

Major Findings:

(1) Hematopoietic Neoplasms - *Leukemias, lymphomas and plasmacytic myelomas* are among the most prevalent neoplasms of man and many species of animals.

Morphologic and epidemiologic characteristics of these neoplasms in man and animals were described in Lingeman, C. H. and Garner, F. M.: *Symposium on Comparative Morphology of Hematopoietic Neoplasms.* National Cancer Institute Monograph No. 32. U.S. Dept. of Health, Education, and Welfare, Public Health Service. Wash., D.C., U.S. Govt. Print. Off., 1969, 365 pp. Subsequent to publication of the Nat. Cancer Inst. Monogr., studies of some selected types of hematopoietic neoplasms of man and animals have continued, including studies of mast cell neoplasms and thymomas of cats and human Hodgkin's Disease and plasmacytic myeloma.

Mast cell neoplasms - Examination of histologic material from the Armed Forces Institute of Pathology's collection and a review of the literature indicates that mast cell neoplasms occur relatively less frequently in cats than in dogs. Unlike dogs, in which most mast cell tumors are relatively benign neoplasms primary in skin, the feline neoplasms more often originate in hematopoietic viscera (although some are primary in skin) and their behavioral characteristics are similar to leukemia or lymphoma.

Thymomas are rare in cats and dogs. A study of two examples of thymomas of cats in the collection of the Armed Forces Institute of Pathology indicates a morphologic similarity to those of man, although neither myasthenia gravis nor other thymoma-associated diseases of man have been observed in cats.

Hodgkin's disease - Because current evidence suggests a role of infectious agents and possibly other environmental agents in causation of Hodgkin's disease (which appears to be an exclusively human disease as yet, no precise morphologic counterpart has been described in any animal) the epidemiology of Hodgkin's disease in an urban community over a 13-year period is being analyzed for evidence of geographic-temporal clustering using a statistical method developed by Mantel and Glass.

Plasmacytic myeloma - Because current evidence suggests that environmental agents are important in the etiology of this neoplasm, the distribution of myeloma is being analyzed in several urban and rural communities for evidence of geographic-temporal clustering. Preliminary evidence indicates an *uneven* distribution in time and space, but further analyses are underway to confirm or refute this impression.

(2) Gastric Adenocarcinomas - These neoplasms are extremely infrequent in animals and are induced by chemical carcinogens only with extreme difficulty. *Dogs* appear to develop spontaneous gastric adenocarcinomas more frequently than other animals. Canine gastric carcinomas are similar to the *diffuse* histologic type of man, rather than the "intestinal" type characteristic of high risk populations. As in man, most arise in the antral segment, along the lesser curvature and metastasize to lymph nodes and liver. They occur in older dogs of both sexes and there is no breed preference. No spontaneous or experimental tumor type appears to have the same pathogenesis as the stomach cancers in Japan, Colombia, or Poland.

(3) Intestinal Adenocarcinomas - Intestinal adenocarcinomas are also infrequent in animals compared to man. They are most frequent in *dogs*, *sheep*, and *hamsters*. *Canine* carcinomas may arise in polypoid adenomas most frequently in the rectum or in the descending colon. They metastasize to lymph nodes and liver. They occur in older dogs and no sex or breed preference has been noted. Contrary to the distribution in man and dogs, carcinomas are more frequent in small intestines in *cats*, *sheep*, and some other animals. Most *feline* intestinal adenocarcinomas originate in the ileum or in the jejunum, occasionally in the large intestines. They vary in degree of differentiation and ability to produce mucin. They metastasize to lymph nodes, liver and other structures. Preneoplastic diseases have been recognized and adenomas are infrequent.

Most *ovine* carcinomas, epidemic in New Zealand and Australia, originate in the jejunum or in the ileum. They are undifferentiated scirrhous adenocarcinomas that metastasize primarily to lymph nodes.

Although spontaneous adenocarcinomas are relatively infrequent in intestines of rats and mice, they can be induced in both small and large intestines of both species by a variety of synthetic and natural chemical substances. Examination of histologic material from rats injected with the intestinal carcinogen dimethylhydrazine (DMH) revealed that this carcinogen can induce a broad spectrum of glandular intestinal neoplasms including adenomas and adenocarcinomas showing all extremes of differentiation and ability to produce mucin and stroma, sometimes in the same animal. While morphologically similar to intestinal adenomas and adenocarcinomas of man, the locations of the experimental neoplasms are different with a larger proportion in the small intestines (mostly in the duodenum). The rat adenomas and adenocarcinomas grow disproportionately larger, relative to the size of the lumens of the intestines, and apparently cause death by intestinal obstruction before metastases occur. Although metastases to lymph nodes and liver do not appear to be as frequent in rats with intestinal carcinomas as in man, special efforts will be made to section mesenteric lymph nodes of experimental animals to confirm or refute this impression.

(4) Mammary Gland Carcinomas - Mammary gland carcinomas are among the prevalent neoplasms in many species of mammals including man, rats, mice, cats, and dogs. They are apparently rare in other rodents, cattle, horses, sheep, pigs and primates. Mammary gland neoplasms of man, mouse, rat, and dog have been well characterized; those of cat have not.

Mammary gland neoplasms are among the most frequent malignant neoplasms of female cats. Preliminary examinations of histologic material from approximately 30 cats with mammary gland neoplasms in the collection of the Armed Forces Institute of Pathology indicate most feline mammary gland neoplasms are ductal adenocarcinomas similar to one human type. Some feline carcinomas form papillary growths. The lungs appear to be most frequent sites of metastases of feline mammary carcinomas although too few lymph nodes were available for assessment of their importance as sites for metastases. Some cats have survived several years following surgical removal of an affected mammary gland.

Although clinical records of most of the Armed Forces Institute of Pathology cases were incomplete regarding whether animal had been spayed, numbers of pregnancies, etc., the occasional occurrence of mammary adenocarcinomas in intact male cats indicates that ovarian hormones are not always essential for their development.

Significance to Biomedical Research and the Program of the Institute:

Because of the importance of environmental factors in causation of human malignant neoplasms, the search for specific causes by epidemiological techniques must also include attention to animals that share man's carcinogenic environment. Animals offer the additional advantage of permitting species-specific experimental testing of suspected carcinogens.

Proposed Course of Project:

To continue to investigate comparative epidemiology of human and animal neoplasms in order to discover factors that may be involved in their etiologies.

As part of the human colon cancer program, efforts are being made to locate material for morphologic and epidemiologic evaluation of two unusual situations that might contribute information to current hypotheses of etiologic mechanisms in colon cancer: (1) primary small intestinal adenocarcinomas and (2) colorectal adenocarcinomas in young people.

Publications

Garner, F. M. and Lingeman, C. H.: Mast cell neoplasms of the domestic cat. *Pathol. Vet.* 7: 517-530, 1970.

Lingeman, C. H.: The challenges of comparative pathology. *Comp. Path. Bull.* 2 (No. 4): 4, 1970.

Lingeman, C. H., Garner, F. M., and Taylor, D. O. N.: Spontaneous gastric adenocarcinomas of dogs. A review. *J. Nat. Cancer Inst.* 47: 137-153, 1971.

Lingeman, C. H. and Garner, F. M.: A comparative study of intestinal adenocarcinomas of animals and man. *J. Nat. Cancer Inst.* 48: 325-346, 1972.

Serial No. NCI-4538
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Epidemiologic Pathology Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Correlations between Trace Metals in Water Supplies in Cancer Mortality

Previous Serial Number: None

Principal Investigator: John Berg, M.D.

Other Investigators: Fred Burbank, M.D. (Epidemiology Branch) and Frank McKay (Biometry Branch), Demography

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.3
Other:	0.2

Project Description

Objectives:

To correlate data on water supply contaminants with geographic variations in mortality from specific cancer types.

Methods Employed:

Various sources of information on water supplies are collected and grouped geographically to coincide with geographic cancer data. Correlations are studied by various computer routines.

Major Findings:

There were no correlations between mortality of any type of cancer and iron, cobalt, or chromium concentrations. Nickel concentrations correlated with mouth and intestinal cancer death rates and arsenic concentrations with mortality from larynx cancer, eye cancer and myeloid leukemia, but these have no biological interpretations. Beryllium, which produces bone cancer in animals, was correlated with bone cancer mortality as well as mortality from breast cancer and uterine cancers but association was weak in subgroups. Lead, a renal carcinogen in animals and a hematopoietic poison was correlated

with mortality from kidney cancer, leukemias, and lymphomas as well as stomach, intestinal, and ovarian cancer. Cadmium concentrations were correlated most closely and most frequently to cancer death rates but the distribution of the metal producing these correlations are not in agreement with other reports.

Significance to Biomedical Research and the Program of the Institute:

Several elements contaminating water supplies are known to be carcinogenic in animal and we are beginning the studies needed to determine whether they play a similar role for humans. These preliminary over views hopefully will lead to more detailed analysis of human intake and animal experiments designed to test human type exposures.

Proposed Course of Project:

Analyses of other metals and correlations with other sources of cancer data continues in our attempt to estimate the importance of establishing protocol investigations of this problem.

Honors and Awards

Invited Speaker, New York Academy of Sciences, Conference on Trace Metals and Diseases, October 1971

Publications

Berg, J. W. and Burbank, F.: Correlations between carcinogenic trace metals in water supplies and cancer mortality. *Ann. N. Y. Acad. Sci.* (In Press).

Serial No. NCI-4763
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on Nuclear Exoribonucleases from Normal and Tumor Cells

Previous Serial Number: Same

Principal Investigator: Michael B. Sporn, M.D.

Other Investigator: Mary S. Baker, M.S.

Cooperating Units: Dr. Robert Gallo, Chemotherapy, NCI
Dr. Anthony Schrecker, Chemotherapy, NCI

Man Years:
Total: 0.7
Professional: 0.5
Other: 0.2

Project Description

Objectives:

To characterize the enzymes of the normal and tumor cell nucleus which are responsible for the *in situ* destruction of the newly synthesized, nascent messenger RNA of the nucleus. To define the role of these enzymes in the cell nucleus, particularly with respect to control of gene action.

Methods Employed:

Highly purified nuclei have been isolated from many cell types, including tracheal epithelial cells, HeLa cells, and Ehrlich ascites tumor cells. The exoribonuclease which degrades messenger RNA in these nuclei has been highly purified. Inhibitors have been tested by classical enzymological methods for their ability to irreversibly inactivate exoribonuclease, without inactivating enzymes of RNA synthesis. Inhibitors of exoribonuclease have also been assayed for inhibition of the RNA-dependent-DNA polymerase (reverse transcriptase) of oncogenic viruses and leukemia cells.

Major Findings:

Several new agents have been tested during the past year and have been found to be potent inhibitors of both nuclear exoribonuclease and reverse transcriptase. The reverse transcriptase assays have been performed by Dr. Anthony Schrecker and Dr. Robert Gallo, NCI. The most active agents have been thymidine-3',5'-di-*m*-bromoacetamidophenylphosphate (TDMB) and 2'-O-methyl uridine-3',5'-di-*m*-bromoacetamidophenylphosphate (Um DMB), both of which give significant inhibition of exoribonuclease at 10^{-5} Molar concentration, and of reverse transcriptase at 10^{-6} Molar concentration. The studies with Um DMB represent the first use of a 2'-O-methyl nucleoside as an active-site-directed irreversible enzyme inhibitor.

Significance to Biomedical Research and the Program of the Institute:

The role of the gene action system is a central problem in all studies of carcinogenesis, whether chemical or viral. Research on the factors which control gene transcription and translation is among the most basic in all of cancer research. Since messenger RNA is the primary product of gene transcription, it is of utmost importance to know the mechanisms which control both its synthesis and degradation. The present project is yielding data on the enzymes and mechanism of messenger RNA destruction in both normal and cancer cells. The testing of potential inhibitors of reverse transcriptase is an area of high priority and relevance in other aspects of the NCI total program.

Proposed Course of Project:

To continue studies on the molecular mechanisms whereby messenger RNA is destroyed in normal cells and cancer cells. To evaluate the role of enzymes of messenger RNA destruction in controlling the growth and differentiation of both normal and cancer cells, and in particular to focus on the effect of carcinogens on the activity of these enzymes. To continue with inhibitor studies on exoribonuclease and reverse transcriptase.

Publications

1. Glinski, R. P. and Sporn, M.B.: Nucleotide synthesis. Derivatives of thymidine containing *m*- and *p*-haloacetamidophenyl phosphate groups. Biochemistry 11: 405-412, 1972.

2. Schrecker, A. W., Sporn, M. B., and Gallo, R. C.: Inhibition of RNA-dependent DNA polymerace by thymidylate derivatives. Cancer Res. (In press)

Serial No. NCI-4697
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Experimental Respiratory Carcinogenesis

Previous Serial Number: Same

Principal Investigator: David G. Kaufman, M.D.

Other Investigators: Curtis C. Harris, M.D., Joseph M. Smith, Valerio Genta, M.D., Michael B. Sporn, M.D., and Umberto Saffiotti, M.D.

Cooperating Units: Dr. Russell Madison and Judith Ober, Microbiological Associates, Inc.

Man Years:

Total:	1.2
Professional:	0.4
Other:	0.8

Project Description

Objectives:

The aim of this project is to elucidate causative factors and pathogenetic mechanisms of respiratory tract cancer and to define biological models for the experimental study of bronchogenic carcinoma.

Methods Employed:

Carcinogens are administered to Syrian hamsters by intratracheal instillations: (a) Benzo[a]pyrene (BP) as a particulate suspension attached to particles of Ferric Oxide; and (b) Nitroso Methyl Urea (NMU) dissolved in buffered saline. In addition to control groups, experimental groups are designed to consider the following points: (a) Tumor yield when benzo[a]pyrene is given at high doses in a short time period; (b) Dose-response studies using single doses of NMU; and (c) Influence of vitamin A deficiency or vitamin A supplimentation on tumor yield where NMU is the respiratory carcinogen. Results are to be derived from analysis of the gross and microscopic lesions observed at death in experimental animals.

Major Findings:

Life-time carcinogenesis studies are still in their early stages and overall conclusions cannot be made from the incomplete results. Preliminary observations indicate that 5 mg of NMU, given as 10 weekly 0.5 mg doses, causes the rapid appearance of respiratory tumors. When animals first receive a course of BP, then later receive NMU, a very high incidence of respiratory tract tumors results. One of these tumors, a metastasizing anaplastic carcinoma, has grown as a transplanted tumor. In experiments designed to determine whether a single dose of NMU is carcinogenic, the first tumor has been observed after 29 weeks.

Significance to Biomedical Research and the Program of the Institute:

These studies are part of the efforts aimed at defining factors leading to the development of bronchogenic carcinoma in animal systems under conditions that resemble those of human lung cancer development. Studies in progress have the following as objectives: (a) Development of a single-dose carcinogenesis system, to permit improved methods for study of tumor promotion and cofactor relationship in respiratory carcinogenesis; (b) To attempt to produce an animal model of bronchogenic small cell anaplastic carcinoma; and (c) To attempt to demonstrate an anticarcinogenic effect of vitamin A in long term carcinogenesis studies where NMU is the carcinogen. These studies coordinate with, or compliment studies in progress both intramurally and in the extramural contract program of the Lung Cancer Segment.

Proposed Course of Project:

Continuation of long-term carcinogenesis studies outlined above as an integral element of the program of the Lung Cancer Segment.

Publications

Saffiotti, U., Montesano, R., Sellakumar, A. R., Cefis, F., and Kaufman, D. G. Respiratory tract carcinogenesis in hamsters induced by different numbers of administrations of benzo[a]pyrene and ferric oxide. Cancer Res. (In press)

Serial No. NCI-4793
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Reports
July 1, 1971 through June 30, 1972

Project Title: *In Vitro* Metabolic Studies in Isolated Hamster Respiratory Tract Tissues

Previous Serial Number: Same

Principal Investigators: David G. Kaufman, M.D., Valerio Genta, M.D., Mary S. Baker, M.S.

Other Investigators: Curtis C. Harris, M.D., Joseph M. Smith, and Michael B. Sporn, M.D.

Cooperating Unit: C. W. Dingman, M.D., Nucleic Acids Section, Chemistry Branch, NCI

Man Years:
Total: 1.4
Professional: 0.8
Other: 0.6

Project Description

Objectives:

The aim of this project is to examine metabolic processes in a respiratory epithelium. These studies are designed to determine how metabolic processes are affected: (1) by chemical carcinogens administered either *in vivo* or *in vitro*, and (2) by vitamin A deficiency.

Methods Employed:

Techniques have been developed for maintaining isolated hamster tracheas *in vitro* as organ cultures for at least four hours. Radioactively labeled precursors for nucleic acid synthesis were added to the *in vitro* incubation medium and were incorporated into the organ fragment. The respiratory epithelium was scraped from the supportive structures of the trachea. Microchemical techniques were developed for isolation of undegraded RNA from the tracheal scrapings. These RNA preparations were examined by acrylamide gel electrophoresis. These methods were employed where chemical carcinogens had been added during *in vitro* incubation, or where the animals from which tracheas were taken were pretreated with carcinogens *in vivo*. In addition, RNA isolated from vitamin

A-deficient animals was examined with these methods and inhibitors of RNA metabolism were employed in efforts to interpret observed changes.

Major Findings:

Previous studies have shown that respiratory epithelium of tracheas maintained *in vitro* in organ culture remain morphologically normal by light and electron microscopy through four hour incubations. RNA has been isolated from normal tracheas subjected to such *in vitro* maintenance for intervals of increasing length in the presence of labeled RNA precursor. The pattern of isotopic incorporation into normal RNA fractions with time was essentially identical to that seen in other tissues. Tracheas from normal animals decreased RNA synthesis, but no qualitative changes in RNA synthesized. RNA synthesized *in vitro* in tracheas from vitamin A-deficient animals showed a selective quantitative change (decrease) in the slowly-migrating, high-molecular-weight RNA species as compared with controls. This alteration was shown to be reversed by vitamin A treatment. Studies are currently in progress using specific inhibitors of RNA metabolism in an effort to determine the nature of this alteration in vitamin A deficiency.

Significance to Biomedical Research and the Program of the Institute:

This project contributes to the part of the lung cancer program, designed to develop biologic models for the study of respiratory carcinogenesis, its causative factors and host control mechanisms. This assay system permits *in vitro* identification of critical biochemical and morphologic alterations which occur in the course of respiratory carcinogenesis induced *in vivo*. The methods developed permit examination of certain aspects of nuclear and cytoplasmic metabolism in viable respiratory epithelial cells. The effects of carcinogens administered *in vitro* can be examined biochemically. Furthermore, these methods will permit direct biochemical study of a respiratory epithelium during the course of *in vivo* carcinogenesis. This work coordinates with the *in vivo* carcinogenesis bioassays being conducted under contracts.

Proposed Course of the Project:

Studies during this project year have concentrated on RNA metabolism in tracheas of vitamin A-deficient animals and in tracheas exposed to carcinogens *in vitro*. During the next year, it is proposed to continue studies to further characterize the altered RNA metabolism in vitamin A-deficient tracheas. In addition to studies of RNA metabolism, it is proposed to examine the binding of carcinogens to DNA and DNA metabolism. Design of these experiments will be such as to exploit observations made in long-term animal studies.

Publications

Kaufman, D. G., Baker, M. S., Smith, J. M., Henderson, W. R., Harris, C. C., Sporn, M. B., and Saffiotti, U.: RNA metabolism in tracheal epithelium: Alteration in vitamin A-deficient hamsters. *Science* (In Press)

Serial No. NCI-4777
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Effects of Carcinogens on Methylation of Nuclear RNA

Previous Serial Number: Same

Principal Investigators: Adhid Al-Arif, Ph.D., Miriam Poirier, M.S., and Michael B. Sporn, M.D.

Other Investigators: Joseph M. Smith

Cooperating Units: None

Man Years:

Total:	2.6
Professional:	1.5
Other:	1.1

Project Description

Objectives:

The nucleolus is known to methylate RNA on the 2'-oxygen of ribose. Since nucleolar lesions are known to be a prominent feature of cells treated with chemical or viral carcinogens, it is important to evaluate whether carcinogens affect 2'-O-methylation of RNA. Failure to methylate RNA would lead to excessive destruction of RNA within the nucleus, and failure to transport RNA to the cytoplasm.

Methods Employed:

Methylation of RNA is being studied in two different systems: (1) the isolated hamster tracheal organ fragment, and (2) isolated rat liver nuclei. New chromatographic methods have been developed to separate methylated nucleosides from normal nucleosides. RNA on acrylamide gels can be quantitatively converted to nucleosides and the extent of methylation measured. A new system for assay of methylation of RNA in vitro by isolated nuclei has also been developed.

Major Findings:

A new paper chromatographic method for the separation of 2'-O-methylated ribonucleosides from non-methylated and base-methylated ribonucleosides, using paper impregnated with ammonium borate and a butanol-borate developing solvent

has been perfected. In this procedure, the four commonly occuring sugar-methylated ribonucleosides, namely 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine are separated from each other. The method can be applied to either in vivo or in vitro studies of RNA metabolism, for the purpose of differentiating between sugar-methylation and base-methylation of RNA. Nuclei isolated from rat liver, when incubated with methyl-labeled S-adenosyl methionine, were found to incorporate label into 2'-O-methyl adenosine, 2'-O-methyl guanosine, 2'-O-methyl uridine, and 2'-O-methyl cytidine of endogenous nuclear RNA. Addition of wheat germ ribosomal RNA to the reaction markedly stimulated 2'-O-methylation of total RNA; in these experiments, the relative incorporation of label into the four 2'-O-methyl nucleosides suggest that 2'-O-methylation of the wheat germ RNA has occured in vitro. These studies are the first to show definitively that 2'-O-methylation of all four ribonucleosides can occur in vitro.

Significance to Biomedical Research and the Program of the Institute:

The problem of stabilization of newly synthesized RNA is a fundamental problem in cell biology and carcinogenesis. The nucleolus is believed to be a key intracellular organelle for RNA stabilization, and 2'-O-methylation of RNA is believed to be a key molecular step to this process in the nucleolus. It is well known that both chemical and viral carcinogens affect nucleolar ultrastructure. The above studies are thus aimed at elucidating a potentially critical molecular lesion in carcinogenesis.

Proposed Course of Project:

To continue as outlined above, and particularly to focus on the effects of different carcinogens on methylation of RNA in the tracheobronchial epithelium. To continue with studies of the effects of carcinogens on 2'-O-methylation of RNA in isolated liver and tracheobronchial nuclei.

Publications

Al-Arif, A. and Sporn, M.B.: An analytical method for the separation of sugar-methylated ribonucleosides from base-methylated and nonmethylated ribonucleosides. Anal. Biochem. (In Press)

Serial No. NCI-4792
1. Office of the Associate Scientific Director for Carcioogenesis, Etiology
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Histogenesis of Squamous Cell Carcinoma of the Hamster Respiratory Tract Caused by Benzo[*a*]pyrene-Ferric Oxide

Previous Serial Number: Same

Principal Investigator: Curtis Harris, M.D.

Other Investigators: Joseph M. Smith, Frank E. Jackson, Paul E. Dedick, Michael B. Sporn, M.D., David G. Kaufman, M.D., Umberto Saffiotti, M.D., Hollis Boren, M.D.

Cooperating Units: Histopathology Unit; Experimental Pathology Branch, NCI; Department of Medicine, Wisconsin School of Medicine, Milwaukee, Wisconsin

Man Years:

Total:	1.0
Professional:	0.4
Other:	0.6

Project Description

Objectives:

Previous investigations of the histogenesis of squamous cell carcinoma in man and animal models have shown that respiratory epithelium becomes hyperplastic, squamous metaplastic and finally neoplastic. These sequential changes in morphology may be better understood and defined by ultrastructural analysis.

Methods Employed:

The method of Saffiotti and co-workers is used to administer intratracheal instillations of benzo[*a*]pyrene carried on ferric oxide particles in hamsters. This treatment produces squamous metaplasia and squamous cell carcinoma in the respiratory epithelium of the Syrian golden hamster. Following single or multiple intratracheal instillations of (1) benzo[*a*]pyrene-ferric oxide; (2) pyrene-ferric oxide; (3) ferric oxide; or (4) saline, hamsters are sacrificed serially up to the time of gross tumor development. Specific segments of the trachea and bronchi are processed for high resolution light and electron microscopic study. Untreated and sham-treated animals are also examined.

Major Findings:

The ultrastructure of the hamster tracheobronchial epitheluum is described in detail for the first time. Acute and chronic effects of the above agents have been studied to date. The acute cellular changes in the tracheobronchial epithelium, produced by intratracheal instillations of benzo[*a*]pyrene carried by ferric oxide particles, included focal replacement of the columnar cells with pleomorphic cells. These had the ultrastructural features of atypical squamous cells, i.e., cytoplasmic filaments, widened intercellular spaces, abnormal desmosomes, and increased number of lysosomal derivatives. Polylobulated nuclei and pleomorphic nucleoli were prominent findings after multiple instillations of benzo[*a*]pyrene-ferric oxide. These atypical squamous cells may show abnormal ciliagenesis or the formation of cytosomes, which are normally found only in the type B alveolar cells. Defects in the basement membrane were also found. Intratracheal instillations of ferric oxide alone or pyrene-ferric oxide produced basal cell hyperplasia, which was reversible. The atypical squamous cells induced by benzo[*a*]pyrene-ferric oxide had ultrastructural features similar to hyperplastic epithelial cells described in the bronchi of smoking dogs and neoplastic squamous cells described in human bronchogenic carcinoma. These atypical squamous metaplasias were persistent. Squamous cell carcinoma are similar in ultrastructure.

Significance to Biomedical Research and the Program of the Institute:

The incidence of bronchogenic squamous cell carcinoma in man has shown a progressive increase during the past five decades. The pathogenesis of bronchogenic carcinoma is being studied in this animal model by several experimental approaches by members of the Lung Cancer Unit. This multifaceted approach allows meaningful correlation of morphological and biochemical changes occurring during carcinogenesis. Our intramural investigations are closely coordinated with the lung cancer Contract Program.

Proposed Course of Project:

The extent and progression of benzo[*a*]pyrene-ferric oxide induced lesions will be quantitated from 1 μ sections of tracheal rings following classification of lesions and their measurement from light micrographs. A serial sacrifice study to determine the effect of hypervitaminosis A on the histogenesis of squamous cell carcinoma has been initiated.

Publications

Harris, C., Sporn, M., Kaufman, D., Smith, J., Baker, M., and Saffiotti, U.: Acute ultrastructural effects of benzo[*a*]pyrene and ferric oxide on the hamster tracheobronchial epithelium. *Cancer Res.* 31: 1977-1989, 1971.

Serial No. NCI-4794
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Autoradiographic Analysis of *In Vitro* Metabolism in Isolated Hamster Respiratory Tract Tissues

Previous Serial Number: Same

Principal Investigators: David G. Kaufman, M.D., Curtis C. Harris, M.D., Joseph M. Smith

Other Investigators: Mary S. Baker, M.S., Valerio Genta, M.D., Michael B. Sporn, M.D., and Frank E. Jackson

Cooperating Units: Dr. Hollis Boren, Marquette School of Medicine; Dr. Russell Madison and Judy Ober, Microbiological Associates

Man Years:

Total:	0.9
Professional:	0.3
Other:	0.6

Project Description

Objectives:

The respiratory epithelium of the trachea is composed of a mixed cellular population. It is the aim of this new project to distinguish the metabolic activities of the various cell types and thus correlate biochemical and morphologic studies in the course of respiratory carcinogenesis. Changes in cellular populations, as well as changes in metabolic activities following carcinogen administration, are examined concurrently.

Methods Employed:

Radioisotopes are incorporated into isolated hamster tracheas *in vitro* using methods developed in this laboratory. Following *in vitro* incubation with isotope, autoradiograms are prepared from semi-thin and ultra-thin sections for light and electron microscopy. Grain counts are then performed on the developed autoradiograms, which distinguish the different cell types and location of grains over nuclei or cytoplasm. The collaboration of Dr. Boren's group is involved in the experimental design, grain counting, statistical analysis and interpretation of results.

Major Findings:

The cellular populations of tracheal epithelium maintained *in vitro* in organ culture incorporate radioactive chemicals from the culture fluid at different rates. Basal cells incorporate lesser quantities of uridine-^{3}H than do ciliated or mucous cells. Ciliated cells do not incorporate thymidine ^{3}H, whereas basal cells and mucous cells do incorporate it. Mucous cells incorporate greater quantities of leucine-^{3}H than do ciliated or basal cells. Tracheas isolated from animals previously treated *in vivo* with Benzo[a]pyrene plus Fe_2O_3 incorporated greater quantities of Benzo[a]pyrene-^{3}H *in vitro* than tracheas from previously untreated animals. Preliminary studies suggest that the greatest proportional difference is the labeling of the heterochromatin areas of the nucleus.

Significance to Biomedical Research and the Program of the Institute:

This project contributes to the part of the lung cancer program designed to develop biologic models for the study of respiratory carcinogenesis, its causative factors and host control mechanisms. This assay system permits *in vitro* identification of critical biochemical and morphologic alterations which occur in the course of respiratory carcinogenesis induced *in vivo*. The methods which have been developed permit some correlation of biochemical observations with respect to constituent cellular populations of this target tissue. Such correlations should be of critical interest where biochemical changes reflect changes in cellular population, such as those known to occur during *in vivo* carcinogenesis.

Proposed Course of the Project:

Future experiments will concentrate on applying these methods to tracheas from animals exposed to carcinogens *in vivo* and *in vitro*. Studies with radioactively labeled Benzo[a]pyrene will be expanded upon, and studies with radioactively labeled Nitroso Methyl Urea will be attempted. These efforts will be directed toward elucidating the distribution of these carcinogens by cell type and by subcellular localization.

Serial No. NCI-4796
1. Office of the Associate Scientific Director for Carcinogenesis
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Squamous Metaplasia of the Hamster Respiratory Epithelium Induced by Vitamin A Deficiency or Benzo[*a*]pyrene-Ferric Oxide

Previous Serial Number: Same

Principal Investigators: Curtis C. Harris, M.D. and Joseph M. Smith

Other Investigators: Michael B. Sporn, M.D., David G. Kaufman, M.D., Frank E. Jackson, and Umberto Saffiotti, M.D.

Cooperating Units: Histopathology Unit, Experimental Pathology Branch, NCI

Man Years:

Total:	0.9
Professional:	0.3
Other:	0.6

Project Description

Objectives:

Mucous and ciliated cells of the respiratory epithelium provide a defense against inhaled pollutants and carcinogens. This columnar epithelium can undergo squamous metaplasia, which may be a morphological stage in the histogenesis of squamous cell carcinoma. It is of interest to compare squamous metaplasia induced by carcinogens to squamous metaplasia found during vitamin A deficiency.

Methods Employed:

Hamsters were maintained on a vitamin A-deficient diet. Animals were examined during 4 stages of deficiency based upon their weight course and clinical signs. Pair-fed animals served as controls. A second group of animals received 10 intratracheal instillations of benzo[*a*]pyrene-ferric oxide. Specific tracheal and bronchial rings were processed for high resolution light and electron microscopic study.

Major Findings:

The sequential changes in the trachea during vitamin A deficiency appear to be: (1) the ciliated and mucous cells flatten; (2) basal cells increase in number; (3) a widened intercellular space develops between the hyperplastic layer of basal cells and the luminal cells are sloughed; (4) basal cells differentiate into flat layers of squamous cells. As described previously by us, intratracheal instillations of benzo[a]pyrene-ferric oxide cause many histological changes including squamous metaplasia with atypical squamous cells. Benzo[a]pyrene-ferric oxide also causes a regular squamous metaplasia which could not be distinguished from squamous metaplasia induced by vitamin A at the light microscopic level, whereas abnormal nucleoli and defects in the basement membrane were apparent at the ultrastructural level. Defects in the basement membrane have been observed in preneoplastic and neoplastic lesions of the skin, manmary gland and larynx.

Significance to Biomedical Research and the Program of the Institute:

Vitamin A has a pivotal role in the control of epithelial differentiation in the respiratory epithelium. The differentiation of basal cells into mucous and ciliated cells is dependent on adequate levels of vitamin A. Vitamin A deficiency results in an alternate path of differentiation, i.e., squamous metaplasia, which also appears to be a preneoplastic stage in the histogenesis of squamous cell carcinoma of the bronchi. Hypervitaminosis A has been found to decrease the incidence of squamous metaplasia and squamous carcinoma in this animal model. Squamous metaplasia induced by benzo[a]pyrene-ferric oxide can be distinguished at the ultrastructural level from squamous metaplasia observed during vitamin A deficiency. This study is designed to contribute to the elucidation of the control mechanisms involved in epithelial differentiation and carcinogenesis.

Proposed Course of Project:

The initial objectives of this project have been completed. A quantitative study to determine changes in cell populations of the respiratory epithelium during hypo- and hyper-vitaminosis A has been initiated. In addition, the toxicity of hypervitaminosis A in hamsters is being studied.

Publications

1. Harris, C. Sporn, M., Kaufman, D., Smith, J., Jackson, F., and Saffiotti, U.: Histogenesis of squamous metaplasia in the hamster tracheal epithelium caused by vitamin A deficiency or by benzo[a]pyrene-ferric oxide. *J. Nat. Cancer Inst.* 48: 743-747, 1972.

Serial No. NCI-4797
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Acute and Chronic Effects of Carcinogenic N-Nitroso Compounds in the Respiratory Epithelium

Previous Serial Number: Same

Principal Investigator: Curtis C. Harris, M.D.

Other Investigators: Michael B. Sporn, M.D., David G. Kaufman, M.D., Joseph M. Smith, Frank E. Jackson, Paul E. Dedick, and Umberto Saffiotti, M.D.

Cooperating Units: Histopathology Unit, Experimental Pathology Branch, NCI

Man Years:

Total:	0.9
Profess onal:	0.3
Other:	0.6

Project Description

Objectives:

N-nitroso compounds, such as diethylnitrosamine (DEN), dimethylnitrosamine (DMN) and N-methyl-N-nitrosourea (NMU) are respiratory carcinogens. NMU is a proximate carcinogen, whereas DMN and DEN require metabolism into active form(s). Intratracheal instillations of NMU induces squamous cell carcinoma of the lung as shown by Herrold. The objectives of this project are: (1) to study the histogenesis of squamous cell carcinoma caused by multiple instillations of NMU and compare these changes with those from a similar histogenesis study using a polynuclear hydrocarbon, benzo[*a*]pyrene; and (2) relate the acute ultrastructural lesions induced by these compounds to changes in RNA metabolism and carcinogenesis.

Methods Employed:

DEN, DMN and NMU are given by parenteral route or by intratracheal instillation. At selected times, animals are sacrificed and tracheal rings are processed for light and electron microscopic examination. The effect of these carcinogens on the incorporation of uridine-H^3 into cellular RNA is being studied by autoradiography, which allows the identification of the various cell populations

in the tracheal epithelium. Multiple instillations of NMU are being given to hamsters for a serial sacrifice study of tumor histogenesis. After the last instillation of NMU, the hamsters are maintained on a normal or hypervitaminosis-A diet.

Major Findings:

DEN, DMN, and NMU all cause acute alterations in nucleolar ultrastructure. Autoradiographic studies have shown that NMU inhibits the incorporation of H^3-uridine in all cell types. Following *in vitro* incubation with NMU, tracheas have been transplanted into hamster cheek pouches to determine whether this short *in vitro* exposure to NMU will cause neoplastic transformation and tumors. The serial sacrifice studies are in progress to study the histogenesis of squamous cell carcinoma. NMU causes a variety of persistant histological lesions containing atypical squamous cells. Enlarged multi-lobulated nuclei and pleomorphic nucleoli are common findings. Autoradiographic studies reveal that these cells incorporate H^3-thymidine more frequently than normal tracheal epithelial cells.

Significance to Biomedical Research and the Program of the Institute:

This project contributes to the part of the lung cancer program designed to develop biologic models for the study of respiratory carcinogenesis, its causative factors and host control mechanisms. N-nitroso compounds are a group of systemic carcinogens, which have marked tissue specificity. Their role in respiratory carcinogenesis is being actively investigated in several projects of the lung cancer program. The morphological and biochemical effects of these agents directly on respiratory epithelia are being compared to those of the carcinogenic polynuclear hydrocarbons.

Proposed Course of Project:

Continuation of the histogenesis studies. The extent and progression of NMU induced lesions will be quantitated from 1 μ sections of tracheal rings following classification of lesions and their measurement from light micrographs.

Serial No. NCI-4798
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Control of Epithelial Cell Differentiation and Carcinogenesis

Previous Serial Number: Same

Principal Investigators: Luigi M. De Luca, Ph.D., and Nancy E. Maestri

Other Investigator: William Henderson

Cooperating Units: Drs. George Wolf and Fabrizio Bonnani, Massachusetts Institute of Technology; Dr. Graham Jamieson, Blood Research Laboratory, The American National Red Cross

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

To study control mechanisms of mucus-squamous cell differentiation by vitamin A and chemical carcinogens in the respiratory tract and in other mucus-secreting and keratinizing tissues.

Methods Employed:

Whole-cell and cell-free systems from hamster tracheal and intestinal epithelia are prepared from vitamin A-deficient hamsters and rats. Free and membrane-bound polyribosomes are prepared by a discontinuous sucrose density gradient. The membrane-bound polyribosomes are used to study *in vitro* synthesis of glycoproteins. To evidence tissue localization of the mucus-component not synthesized in vitamin A-deficiency, an antiserum against the purified component is prepared in chickens and the antigen is localized by indirect immunofluorescence. The synthesis of membrane-bound glycolipids functioning as intermediates in the biosynthesis of glycoproteins is investigated in the trachea by extracting with chloroform-methanol (2:1) the synthesized labelled glycolipids and purifying them on columns of DEAE-cellulose and Silicic acid.

Major Findings:

By using labeled D-Fucose, D-Mannose, and D-Glucosamine, or their nucleotide derivatives, it was found that the biosynthesis of a specific glycopeptide was decreased during keratinization of hamster and rat tracheal epithelium caused by vitamin A deficiency. By PAS and immunofluorescence staining, it was found that tracheal glands of vitamin A deficient rats were greatly reduced in number in A deficiency. This is in accord with the lack of synthesis of the specific glycoprotein. Preliminary data indicate that the vitamin may be acting within the rough endoplasmic reticulum matrix to allow biosynthesis of mucus components by transferring mono- or oligosaccharides onto newly synthesized polypeptides.

Significance to Biomedical Research and the Program of the Institute:

This project contributes to the identification of key control mechanisms of carcinogenesis: it is a part of the lung cancer program, addressed to the development of mechanisms of inhibition and control of respiratory carcinogenesis. The control of mucus cell differentiation by vitamin A is also of great interest in helping to understand the mechanism of carcinogenesis in epithelial tissues in general. The vitamin allows formation of goblet cells and its absence leads to hyperplasia of basal cells, squamous metaplasia and keratinization: a sequence of events similar to what takes place in the respiratory epithelium after exposure to benzopyrene. The fucose-glycopeptide, shown to be present in goblet cells and in glands of the respiratory and intestinal epithelium, can be used as a specific marker for differentiative responses in epithelial tissues. The immunofluorescence assay will be of help in these studies.

Proposed Course of Project:

To continue studies as outlined above.

Publications

1. De Luca, L., Anderson, G. H., and Wolf, G.: The *in vivo* and *in vitro* biosynthesis of lung tissue glycopeptides. *Arch. Intern. Med.* 127: 853-857, 1971.

2. De Luca, L., Kleinman, H. K., Little, E. P., and Wolf, G.: RNA metabolism in rat intestinal mucose of normal and vitamin A-deficient rats. *Arch Biochem.* 145: 332-337, 1971.

3. De Luca, L., Schumacher, M, and Nelson, D. P.: Localization of the retinol-dependent fucose-glycopeptide in the goblet cell of the rat small intestine. *J. Biol. Chem.* 246: 5762-5765, 1971.

Serial No. NCI-4699
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Program and Data Analysis Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Carcinogenesis Bioassay Data System

Previous Serial Number: Same

Principal Investigators: Norbert P. Page, D.V.M.; John A. Cooper, II, Ph.D.; Sidney Siegel, Ph.D.; Umberto Saffiotti, M.D.; and Ursula Evans

Other Investigators: Larry Martin, Mary Linhart, Joe Campbell, and William Vincent, Data Management Branch, Division of Computer Research and Technology, NIH

Cooperating Units: None

Man Years:
Total: 3.20
Professional: 2.05
Other: 1.50

Project Description

Objectives:

To develop and implement a comprehensive information system for collection, storage, manipulation, recall and analysis of selected data pertaining to carcinogenesis bioassay studies. In so doing, to create and maintain a file of data about substances studied as part of the Chemical Carcinogenesis Program.

Methods Employed:

Standard systems design methods, automated data processing techniques, as well as the NIH/DCRT hardware and software environment have been utilized in the development of the operational system. The essential data elements to be collected have been identified and forms constructed for the collection of this data. These forms have been field tested and after modifications are now being used for the introduction of real data into the data system. The eight types of input forms correspond respectively to the following experimental components about which data are collected: Chemical Batch, Treatment or Preparation, Animal Colony

Animal Group, Experimental Environment, Cage Observations, Group Observations, and Individual Animal Pathology Record. A chemical data subsystem has been merged into the CBDS. The above contains the following data on substances under investigation:

1. Name (Chemical Abstracts preferred, inverted form)
2. Synonyms (common and trade names)
3. Wiswesser Line Notation
4. Production data
5. Manufacturer
6. Fragment codes
7. Chemical Abstracts Registry Number
8. Molecular Formula

Major Findings:

During the past year, the Carcinogenesis Bioassay Data System (CBDS) has moved from the developmental to the implementation stage. Data are now being collected from the contractors who have initiated new studies during fiscal year 1972. In addition, a few studies begun prior to July 1971 have also been entered into the system. As the result of minor difficulties experienced by the contractors, the data input forms and specific programs are being continually modified as additional experience is gained. Data output in the form of summary reports and graphs are now being produced from the stored data.

Significance to Biomedical Research and the Program of the Institute:

Because of the size, diversity and complexity of the Carcinogenesis Bioassay Program, it is essential that a method be available to manage and make optimal use of the information generated by its various activities. The Carcinogenesis Bioassay Data System will provide this and will, in addition contribute to uniformity of reporting results of bioassay studies and to standardization of chemical and tumor nomenclature. It will also provide for routine rapid evaluation of bioassay studies, including comparison of results from multiple sources, and for ready access to summary data for statistical analysis.

Proposed Course of Project:

All new bioassay studies will be entered into the system as they are initiated. During fiscal year 1973, the developmental aspects will move further into Phase II, the programming of sophisticated data analysis. This will be accomplished by the increased interaction of DCRT and Carcinogenesis staff as well as the utilization of expert advice from persons experienced in the analysis of such data.

Serial No. NCI-4539
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Program and Data Analysis Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Information Dissemination for the Carcinogenesis Program

Previous Serial Number: None

Principal Investigators: Sidney Siegel, Ph.D.; John A. Cooper, II, Ph.D.; Norbert Page, D.V.M.; Terry Kuch, Elizabeth Weisburger, Ph.D.; Umberto Saffiotti, M.D.

Other Investigators: Hilary Burton, National Agricultural Library (Data Systems Application Division)

Cooperating Units: None

Man Years:

Total:	1.6
Professional:	0.6
Other:	1.0

Project Description

Objectives:

To develop, implement and maintain systems which gather, catalogue and then disseminate in a timely manner information relevant to the Carcinogenesis Program of the Institute.

In so doing, create data bases which will enable scientists from the broad spectrum of disciplines comprising carcinogenesis research to be made aware of information both directly and indirectly pertinent to their own efforts.

Methods Employed:

(1) Production by contract of the periodic publication "Carcinogenesis Abstracts" which results from scanning approximately 3,500 different biomedical periodicals for articles dealing with:

a) Reviews
b) Chemical Carcinogenesis
c) Physical Carcinogenesis

d) Viral Carcinogenesis
e) Immunology
f) Pathogenesis
g) Epidemiology and Biometry

(2) Contracts are in effect for continuing the series PHS #149, "Survey of Compounds Which Have Been Tested For Carcinogenic Activity". Approximately 4,000 different periodicals were scanned for articles relevant for this publication. Literature from 1961-1967 and 1970-1971 is now being processed and will be available as separate volumes November, 1972 and February, 1973 respectively. The volume for the 1968-1969 literature is available and in the process of being distributed.

(3) Interest profiles which automatically examine data bases have been developed to disseminate selective information to program scientists.

(4) Functioning as a clearinghouse for information requests relevant to the program. In so doing establish and maintain data bases needed to respond to these requests.

(5) Establishing critical analyses of the Biomedical literature concerning topics of interest for the Carcinogenesis Program, by helping to define the problem, establish the necessary literature, screening, extraction and evaluation teams.

Major Findings:

During the past year the Carcinogenesis Program has continued the formal production and distribution of both Carcinogenesis Abstracts and PHS publication #149.

A study of literature activity by the contractor concerned with Carcinogenesis Abstracts has demonstrated a significant increase in relevant information. The increase reflects a higher level of activity and funding in this area of research.

Subscription lists along with requests for both publications and blocks of information extracted from these publications continues to expand.

Significance to Biomedical Research and the Program of the Institute.

Development, implementation and then monitoring of a research program requires a flow of as much information as possible. This information must be grouped or indexed in a logical manner so that the data bases built to support it can be searched and/or manipulated in a logical manner. Once this is accomplished, what is available can be annotated with comments concerning the reliability of the data.

Information projects which are now on-going combined with those ready for implementation are designed to interface the scientific and administrative information needs of the Carcinogenesis Program.

information needs of the Carcinogenesis Program.

Proposed Course of the Project:

The project will continue to monitor its formal information publications and suggest restructuring if the direction of research changes enough that other formats must be considered.

The project will continue to be sensitive to the general and specific information needs of the program. Information systems to be generated will be so structured that they will be able to respond to the ever changing profile of research. They will be able to store what is new and different with more conventionally formatted data and still not lose ability to search and retrieve both kinds of information.

Honors and Awards

Manager, Information and Resources Segment, Carcinogenesis, Etiology, NCI

Member, Information and Resources Segment Core Working Group.

Participant, ad hoc Group to evaluate--Carcinogenesis Bioassay of Nitrilotriacetic Acid (NTA); Report submitted to the Surgeon General, USPHS, September 1971

Serial No. NCI-4540
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Program and Data Analysis Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Radiation Carcinogenesis Program

Previous Serial Number: None

Principal Investigator: Norbert P. Page D.V.M., M.S.

Other Investigator: Marvin Schneiderman, Ph.D., Demography Area, Etiology, NCI

Cooperating Units: Atomic Energy Commission; Bureau of Radiological Health, FDA, HEW; Environmental Protection Agency

Man Years:

Total:	0.75
Professional:	0.50
Other:	0.25

PROJECT DESCRIPTION

Objectives:

To determine the state of knowledge and to develop and supervise a program directed toward the following specific aspects of radiation carcinogenesis: a) interactions and potential synergisms with chemical and biological agents, b) assessment of important biological and physical variables that might influence tumorgenesis with radiation, for example, age at time of exposure, microbial status, immune deficiency, and type radiation; and c) mechanisms of action.

Methods Employed:

The current status of research in radiation carcinogenesis is being determined by a review of literature and research project reports of various laboratories and agencies that support radiation carcinogenesis research.

Discussions are held with scientists who are employed in this area of research, either when they are in Bethesda or when visits can be conveniently made to their laboratory in conjunction with other trips.

The principal investigator remains informed of research supported by other agencies by participating in reviews or scientific evaluations of on-going or proposed research programs. He also acts as project officer for those contracts in the Carcinogenesis Area in which radiation is being studied as a carcinogen. The Demography Area continues to support a major effort with the Atomic Bomb Casualty Commission on the late effects of radiation, including radiation carcinogenesis. Information on that project is reported in the Demography Section.

Major Findings:

Summaries of on-going radiation carcinogenesis research have been obtained through the Science Information Exchange (S.I.E.) of the Smithsonian Institute. Contacts have been made with other government agencies to receive annual progress reports as well as other information pertaining to radiation carcinogenesis research.

A meeting has been held with members of the Bureau of Radiological Health (FDA) and the Environmental Protection Agency. At this meeting representatives of the three agencies presented discussions of their research and interest in the epidemiological aspects of radiation carcinogenesis. While the feasibility of cooperative efforts was discussed at this meeting no joint efforts have resulted yet. As another activity, position papers as well as replies to inquiries regarding radiation carcinogenesis were prepared during the past year.

It is evident that while much research has been conducted on the hazards of radiation, little is actually known about the mechanisms by which radiation exposure can result in cancer and of the interactions of radiation with other agents. A large amount of work supported by other agencies has been with experiments using large numbers of animals to determine dose-response relationships. The effort directed toward understanding mechanisms has dealt heavily with recovery parameters. While other aspects have been explored, the interactions as well as recent developments in non-radiation carcinogenesis, e.g., reverse transcriptase, have not been adequately evaluated.

Current controversy regarding the hazards, from low levels of radiation for which the animal experiments are not adequate to assess, may be resolved when mechanisms of carcinogenesis are clarified. Efforts at the in vitro transformation by use of radiation have been rather limited and may be a useful area for additional work.

Significance to Bio-Medical Research in the Program of the Institute:

The National Cancer Institute is concerned with carcinogenesis regardless of the etiological agent. The Institute has not supported a large program in this area due to the commitment other agencies have made in this regard. The mechanisms by which environmental agents result in cancer induction may be quite similar for all etiological agents. Radiation represents a research tool which is easy to use compared with chemical or biological agents. Understanding radiation carcinogenesis may be important in developing an understanding of carcinogenesis induced by other etiological agents. From the hazards view-point, radionuclides have been demonstrated to be a potent class of carcinogens and represents a risk to mankind that must be adequately evaluated. The expertise available in the NCI Intramural and Contract Programs, could provide important direction in the assessment of a radiation hazard. With the introduction of new materials, for example, the neutron-emitter Californium-252 as an isotope useful in bio-medical research and industrial applications, additional research is required to assess their potential hazards.

Proposed Course of Project:

It is expected that liaison activities and exchange of information with other agencies will be further developed during the coming year. Attempts will be made to establish liaison with laboratories in foreign countries also engaged in radiation carcinogenesis research. It is anticipated that a workshop will be held on the mechanisms of action and interactions of radiation and other agents.

Honors and Awards

Chairman, Interagency Meeting on Radiation Carcinogenesis Studies, NCI, Environmental Protection Agency, Bureau of Radiological Health, September 22, 1971

Manager, Lung Cancer Segment Advisory Group, Carcinogenesis, NCI

Member, Information and Resources Advisory Group, Carcinogenesis, NCI

Member, Etiology Interprogram Group, NCI

Member, Civil Defense Committee on Vulnerability of Food Crops and Livestock Production to Fallout Radiation

Member, Ad Hoc Review Group on Concepts and Techniques in Pathology Applicable to Assessment of Long-Term, Low-Dosage Toxicity Studies in Animals for National Center for Toxicology Research, Federation of American Societies for Experimental Biology

Lecturer, Armed Forces Radiobiology Research Institute, Bethesda, Maryland, January 1972

Participant, Program Review, Lovelace Foundation for Medical Education and Research (AEC), Albuquerque, New Mexico, January 1972

Invited Participant, Meeting of International Radiation Protection Association, Budapest, Hungary, May 3-5, 1972

Participant, Ad Hoc Group to Evaluate Carcinogenesis Bioassay of Nitrilotriacetic Acid (NTA); Report Submitted to the Surgeon General, USPHS, September 1971

Publications

Page, N. P., Ainsworth, E. J., Taylor, J. F., and Leong, G. F.: Recovery of sheep after whole-body irradiation: A comparison of changes in radiosensitivity after acute or protracted exposure. Radiat. Res. 46: 301-316, 1971.

Taylor, J. F., Still, E. T., Page, N. P., Leong, G. F., and Ainsworth, E. J.: Acute lethality and recovery of goats after 1 MVp X-irradiation. Radiat. Res. 45: 120-126, 1971.

Page, N. P. and Still, E. T.: Factors modifying the response of large animals to low-intensity radiation exposure. In Warman, E. A. (ED): Proceeding of the National Symposium on Natural and Manmade Radiation in Space. Las Vegas, Nevada, National Aeronautics and Space Administration, Technical Memorandum X-2440, 1972, pp. 622-632.

Serial No. NCI-4540

Page, N. P. and Still, E. T.: Vulnerability of livestock to fallout gamma radiation. In Bensen, D. W. and Sparrow, A. H. (EDS): Survival of Food Crops and Livestock in the Event of Nuclear War. Atomic Energy Commission Symposium Series, No. 24 (Conf-700909). Oak Ridge, Tennessee, U.S. Atomic Energy Commission, Division of Technical Information, December 1970, pp. 648-653.

Serial No. NCI-4541
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Program and Data Analysis Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Management of Animal Resources

Previous Serial Number: NCI-4799

Principal Investigators: Norbert P. Page, D.V.M. and Raymond F. Newell

Other Investigators: Samuel Poiley, Mammalian Genetics and Animal Production Section, Chemotherapy, and Mark Conner, Ph.D., Institution Compliance Section, DRG

Cooperating Units: NCI, Institute of Laboratory Animal Resources, NAS, and NIH Animal Resources Groups.

Man Years:

Total:	1.0
Professional:	0.8
Other:	0.2

Project Description

Objectives:

(1) Plan and effect procurement of animals used in the Carcinogenesis Intramural and Contract Programs; (2) Assure compliance with the Animal Welfare Laws and the NIH Guide for Laboratory Animal Facilities and Care; (3) Plan and support the development of animal models for cancer research as well as to collect and provide information on laboratory animals.

Methods Employed:

Animal requirements for the Carcinogenesis Program are determined as far in advance as possible. These are then presented to the Mammalian Genetics and Animal Production Section for procurement from NIH or contract animal suppliers. Carcinogenesis provides financial support for this operation by contract with the suppliers.

An NCI ad hoc group on animal welfare has been established to assure

compliance with the Animal Welfare Laws (PL-89-544 and PL-91-579) and NIH guide on construction and operation of animal facilities (PHS Publication No. 1024). All contractors have been notified to submit assurances of compliance with the Animal Welfare Laws and NIH standards.

Discussions are held with the Contracting Office and legal counsel as necessary regarding the legal requirements. Visits are made to current or proposed contractors as well as to intramural facilities to determine adequacy of animal care. Consultation service is provided as desired. Surveys are being made by the NCI ad hoc group of all NCI animal facilities and operations to determine measures needed to achieve maximum efficiency and quality of animal care.

Characteristics and availability of animals are obtained to provide better information to the carcinogenesis scientists for selection of appropriate animal models. Support and guidance for the laboratory adaptation and breeding of new species or strains which have potential usefulness as animal models for cancer research is an important aspect of this project. Discussions are held with the NIH Animal Resource Groups, and the Institute of Laboratory Resources, National Academy of Science, as well as other scientists as needed.

Major Findings:

An expansion of the bioassay program resulted in a significant increase in animal requirements. Unfortunately the animal contractors were unable to expand their operations rapidly enough to meet the increased demand and it was not possible to make deliveries as requested.

Meetings of the NCI ad hoc Group on Animal Welfare have been held to implement the provisions of PL-91-579 in the Contract and Intramural programs. In so doing, the Group has worked closely with Dr. Conner of the Institution Compliance Section (DRG).

Animals are selected for the Carcinogenesis Program on the basis of certain desirable characteristics. Often, however, information on normal survival, spontaneous incidence of tumors and insidious medical problems is not readily available from the animal supplier. This occasionally results in using an animal whose undesirable traits outweigh its beneficial characteristics. The wild European hamster (Cricetus cricetus) develops bronchogenic squamous cell carcinoma similar to that seen in man and could be a useful animal model. Partial success has been obtained in the capture, adaptation and breeding of this species in the laboratory. (Medizinische Hochschule, Hannover).

Significance to Biomedical Research and the Program of the Institute:

The quality of animal research depends greatly upon the animal model selected and their health and care in the laboratory. Adequate disease control, diet, and freedom from stressful conditions are especially

important for carcinogenesis research in which animals are maintained for their lifespan. Animals dying from non-experimental treatment midway or late in a carcinogenesis experiment represents a significant loss in time and money. The information on species now available as well as the development of new models will be made available to all programs of NCI as well as to the scientific community at large.

Although the chairman and one member of the NCI ad hoc Group on Animal Welfare are in the Carcinogenesis Program, the Group functions for all programs within NCI and could serve as an example for the other institutes in fulfilling their obligation to the Animal Welfare Laws.

Proposed Course of the Project:

Alternative methods to the current system for meeting the program needs for animals will be explored. The adequacy of animal care and adherence to NIH and legal standards by both contractor and intramural programs will be further assessed during the next year and assistance will be given where needed. It is expected that considerably more attention will be given to the collection and dissemination of information on laboratory animals. Cooperation and advice will be given to the Institute of Laboratory Animal Resources (NAS) in a computerized information storage and retrieval system for species and strain information.

Honors and Awards

Chairman, NCI Ad Hoc Group on Animal Welfare

SUMMARY REPORT
Biology Branch
July 1, 1971 - June 30, 1972

The mission of the Biology Branch is to apply knowledge in the fields of Cell Biology and Immunology to the solution of problems in chemical carcinogenesis that may contribute to the control of cancer in man. The Biology Branch was created in 1966 when the Immunochemistry Section, General Laboratories and Clinics was transferred to Etiology and merged with elements of the Carcinogenesis Studies Branch. Subsequently, several members of the Biology Branch formed the nucleus of the Experimental Pathology Branch, established in 1969. The Biology Branch now consists of the following sections: Cellular Immunity, Cytogenetics and Cytology, Immunochemistry and the Molecular Separations Unit.

Major effort is expended in studies on the relationship between immunity and chemical carcinogenesis. Within this program, the Biology Branch attempts to maintain an optimal balance between applied and basic research. Members of the Biology Branch, consisting of Ph.Ds. and M.Ds., constitute a spectrum ranging from post-doctoral trainees to established investigators. Many of these investigators who have completed their training in the Biology Branch now occupy important positions and are instrumental in demonstrating to the biomedical community the rationale of the goal-oriented, National Cancer Institute approach to the cancer problem.

Of the many goals contained in the various phases of the plan for the Chemical Carcinogenesis Program, members of the Biology Branch have focused on the following:

1. Identification of population groups exposed to known carcinogens in their environment. This approach takes advantage of the fact that several chemical carcinogens are also allergens. The immune response to these allergens (haptens) is being studied as a possible means of determining that individuals have been exposed to them.

2. Identification and selection of chemicals for bioassay of their carcinogenic potential. The rate of introduction into the environment of new compounds that are potentially carcinogenic is so great that the exclusive use of animals for their bioassay is prohibitive. Therefore, high priority has been given to the development of quantitative *in vitro* bioassays to determine carcinogenic transformation of normal to malignant cells as a screen to select those agents to be assayed in animals. Procedures are now ready for preliminary field tests.

3. Development of measures to prevent or modify tumor growth. Methods are now available to eliminate established tumors in man and animals by taking advantage of "non-specific" components of the immune system. These methods are being studied to determine how early in the carcinogenic process these methods can be used to correct or modify the state leading to tumorigenesis.

4. Application of corrective measures in human subjects. Certain forms of human cancer are being successfully treated by immunologic methods. The

methods we have used in animal models are similar to those being used for cancer patients. On the basis of our guinea pig model experiments, we have been able to offer a rational approach to this form of therapy in the case of cancers in or near the skin. Studies are under way to determine how early in the carcinogenic process the effective corrective measures can be applied.

Highlights of Current Achievements in the Biology Branch

1. Until recently antitumor effects achieved by the administration of Mycobacterium bovis (strain BCG) to guinea pigs required the use of living organisms. The need for living BCG has now been circumvented. BCG cell walls attached to oil droplets are as effective in the prevention and treatment of guinea pig cancer as living BCG.

2. Guinea pig tumors were found to contain at least two types of surface antigens not found on normal guinea pig cells. One of these types of antigens is individually specific for each tumor line. The other type of antigen is shared among the tumor lines and with an antigen on normal guinea pig embryo cells. Cytotoxic antisera specific for each of two tumor lines have been prepared. Immunization of guinea pigs with soluble individually specific tumor antigen causes the acquisition of tumor specific immunity.

3. Treatment of hamster embryo cells in culture by x-irradiation enhances the transformation of these cells by chemical carcinogens. Transformation of and toxicity to hamster embryo cells in culture have been shown to be separate functions of the chemical carcinogens benzo(a)pyrene and 3-methylcholanthrene. Carcinogens which have not been demonstrated to produce transformation directly in vitro (probably because they were not properly metabolized in vitro), were injected into pregnant hamsters. Mass in vitro cultures of fetal cells from hamster embryos treated in this way yielded a highly significant incidence of transformation.

Cellular Immunity Section

Studies in this Section continue to be aimed at establishing the mechanism of and optimal conditions for tumor prevention and treatment by BCG. A characteristic feature of tumor destruction by living BCG in vivo is the presence of large numbers of histiocytes at the primary tumor site and at metastatic sites such as regional lymph nodes. Agents other than living BCG that produce cell-mediated immune responses (vaccinia virus, oxazolone) either had no effect on or enhanced the growth of tumor. The requirement for living BCG has been circumvented by the use of BCG cell walls attached to oil droplets. This is important because if BCG becomes widely used for the prevention and treatment of cancer it would be preferable not to expose cancer patients to the risks that may accompany infection with living BCG. Other requirements for BCG-mediated destruction of tumor transplants and their metastases in guinea pigs are 1) a host capable of developing a cellular immune response to BCG, 2) an adequate dose of BCG, 3) injection of BCG directly into the skin tumor transplant and 4) a tumor mass weighing 100 mg or less.

Tumor destruction by BCG has been demonstrated in the mouse, guinea pig and man. In the guinea pig, destruction of tumors by BCG is usually followed by the appearance of lymphocyte-mediated, specific antitumor immunity. In the mouse,

however, destruction of certain tumors can occur without the development of specific tumor immunity.

A vaccine consisting of living BCG and tumor cells was tested for its capacity to produce specific antitumor immunity in guinea pigs. This vaccine, administered intradermally, prevented the growth of tumor at an intradermal challenge dose of 10^6 cells given on the day of vaccination but did not prevent the growth of a challenge dose of 10^7 cells. Tumor cells in the vaccine occasionally produce tumors; this can be prevented, without loss of immunogenicity by x-irradiation of the tumor cells before they are mixed with BCG.

Reliable in vitro tests of specific cell-mediated tumor immunity have been developed. These tests are based on the blastogenic transformation of lymphocytes and on the inhibition of macrophage migration. This is important because the availability of such tests will make it possible to evaluate the tumor immune status of patients with cancer.

Cytogenetics and Cytology Section

The members of the Cytogenetics and Cytology Section study the process of chemical carcinogenesis in order to develop in vitro bioassays for the detection of carcinogenic agents in man's environment. The immediate aim of the studies is to use in vitro techniques to determine conditions which influence the morphologic, immunologic, and pathologic changes that characterize transformation of cells from the normal to the cancerous state. These studies have dealt with the effects of chemical carcinogens, non-carcinogens, viruses, and irradiation on cells in culture. Hamster embryo cells have been used in most of these studies. Attempts are now being made to use guinea pig and human cells.

An understanding of the significance of chemical transformation of cells by a variety of compounds requires evidence that toxicity and transformation are separable factors. This is particularly important when one is studying normal cells in culture since only a small proportion of the cells are able to grow as discrete colonies. Of those cells that are able to grow, many are either normally sensitive to the toxicity of the chemical and thus are eliminated or resistant to the toxicity and incapable of being transformed; only a small proportion of cells can undergo in vitro transformation. Our studies have shown that hamster cells that were plated to form colonies and treated with benz(a)anthracene or 7, 8-benzoflavone prior to the addition of carcinogen were protected from the toxicity normally associated with benzo(a)pyrene or 3-methylcholanthrene. Furthermore, in addition to the protection from the toxic action of the potent carcinogens, there was a demonstrable increase in the rate of transformation when calculated on either a per cell or colony basis. Thus, it was clearly demonstrated that the toxic and transforming activity of carcinogenic polycyclic hydrocarbons are dissociable and that the two processes as has been suggested are different.

X-irradiation of hamster cells prior to exposure to chemical carcinogens has resulted in an enhancement of transformation. As a continuation of this study we have investigated further the time relationship between irradiation and carcinogen exposure. Frequency of transformation is proportional to concentration of carcinogen. The increased transformation frequency observed

appears to be time related; maximum enchancement is found if carcinogen is added 48 hr subsequent to irradiating the cells. Subsequently, there is rapid loss of sensitivity which indicated that irradiation does not simply select for pre-existing cells genetically sensitive to transformation. There may still be temporary physiological states which are radiation resistant and highly sensitive to transformation, though it seems unlikely that these conditions would persist for at least two days. It is more reasonable to conclude that the increased sensitivity to transformation is due to direct action of irradiation on the cells. The study on interaction of chemical and physical agents is extremely important to the study of the causes of carcinogenic effects on a cell. It is important to determine whether radiation causes a somatic mutation which expresses itself as a neoplastic transformation or whether radiation has an indirect effect which facilitates the process of carcinogenesis. Radiation, either by x-irradiation or ultraviolet light has in this system failed to produce recognizable transformation.

Investigation of the processes by which chemical carcinogens interact with cell receptors is of paramount importance in determining the significant stages of chemical carcinogenesis. Using our hamster systems, we have been able to demonstrate that close to 100% of the cells in culture were capable of incorporating radio-active carcinogen. When carcinogen and DNA inhibitors were used, there was no alteration of binding pattern of tritiated carcinogen incorporation in nuclei. RNA inhibitors such as Actonomycin D, and 2-mercapto-1-β-4-pyridethy-benzimidazole were capable of altering the binding pattern of carcinogen and caused partial inhibition of thymidine-^{3}H inhibition, but with the RNA inhibitors much more toxicity was noted than with the DNA inhibitors. Protein inhibitors, chloramphenicol and cyclohexamide partially inhibited the toxicity of 7, 12-dimethylbenz(a)anthracene and the incorporation of the tritiated carcinogen. The question remains what is the relationship of the depression of toxic effect and the reduction of binding; is it due to inhibition of reactions involving the active metabolite of the carcinogen with the protein, or is it an indication that another cell component is actually involved?

It is accepted that metabolic conversion to reactive intermediates is a prerequisite for carcinogenic activity for many tumor producing compounds. Many of these reactive compounds are mutagenic to bacteria and phage. Transformation obtained with a series of aromatic amines present an interesting situation. N-2-flourenylacetamide (FAA) is slightly active as a transforming agent after seven days on cells, N-hydroxy FAA is similarly active, while the acetoxy-FAA is highly active as a transforming agent. Because of recent developments for measuring drug resistance ("Mutagenicity") in mammalian cell culture, we compared transformation of Syrian hamster cells and mutagenicity of Chinese hamster cells under similar experimental conditions. After 4 hrs. treatment with test compound and 12-16 days with 8-azaguanine, mutation frequency was also greatest with N-acetoxy FAA; however, with 100 μg N hydroxy FAA/ml medium significant transformation did occur. The latter may reflect differences in ability of cells to metabolize the N hydroxy FAA. The positive correlation between mutagenicity and transformation is intersting and raise the question concerning involvement of somatic mutation. It needs to be shown that both phenomena occur at the same specific site.

We have reported induction of transformation in Syrian hamster cells by carcinogenic chemicals of different classes. However, chemicals such as urethan and diethylnitrosamine, known to be potent carcinogens in vivo, produced no transformation when applied to cells plated to produce discrete colonies. To investigate possible in vivo activation and to minimize false negatives, we modified the bioassay system with intraperitoneal injections of the chemical (mg/100 g body wt.) into pregnant hamsters on day 12 of gestation and removal of fetuses on day 14 to provide time for completion of metabolic interaction. When the cells were prepared as usual and cloned from a secondary or tertiary culture on an irradiated feeder layer without additional chemical, transformation was extremely significant. Transformed colonies resulted from injections of 7 carcinogens at concentrations non-lethal to fetuses: urethan (1 mg.), and diethylnitrosamine (0.2 mg.), ethylnitrosourea (0.5 mg.), 4 nitroquinoline-N-oxide (1 mg.), aflatoxin (0.1 mg.); benzo(a)pyrene (0.5 mg.), 3-methylcholanthrene (1 mg.), and 7-12 dimethylbenz(a)anthracene (1 mg.). The cloning efficiency (8-12%) indicated no significant toxicity relative to untreated cultures. Examination of colonies obtained from later passages showed increased transformation frequency. Cells derived from transformed colonies produced fibrosarcomas when injected into irradiated hamsters. Therefore, transformation by chemicals that require activation and by those that act directly on cells can be demonstrated in vitro by injection of chemicals in pregnant animals during specific gestation periods.

In vitro chemical carcinogenesis should be able to provide many different models for bioassay of chemicals and for investigating genetic and metastatic problems. Epithelial cells, particularly if obtained from a differentiated tissue such as liver, would make possible significant comparisons between epithelial and fibroblast-like transformation. We have investigated a rat liver cell line which probably consists of differentiated parenchymal cells. The cells of this line have a number of biochemical and morphologic attributes of normal liver parenchymal cells and previously were considered non-transformed. We found that these cells when injected into irradiated rats produced a Reuber type hepatoma. Furthermore, these cells were refractory to in vitro treatment with N-2-fluorenylacetamide. These experiments demonstrate that it is possible to obtain carcinoma related to liver from normal liver cells that have been in culture. They also show that cell lines must be examined critically if they are to be used for bioassay.

Immunochemistry Section

Members of the Immunochemistry Section continue to develop an integrated program in which results of investigations of the basic mechanism of immune cytotoxic reactions are applied to the target oriented program of the Section and other areas of the Biology Branch.

Our understanding of how complement components interact with one another and with cell surfaces carrying antibodies has broadened. The first component of complement is the component that interacts with antibody-antigen complexes and gets activated by them. The active C1 then in turn activates the next two components in this sequence and thereby starts off a reaction sequence producing a wide variety of biological and pharmacological activities. We have found that treatment of serum containing precursor C1 with chloroform or ether results in an activated C1. None of the other organic solvents tried produced this effect. In studying the mechanism of activation of C1 by antibody-antigen

complexes we showed that binding of C1 is followed by three internal activation steps, the last one of which yields the active form of the molecule. The internal activation steps seem to be enzymic in nature. We also found that trypsin is capable of activating C1. Thus certain lipid solvents, enzymes and antibody-antigen complexes induce activation of the molecule. Presently we are tieing together these observations to obtain a rational theory for the process of activation.

The fixation by and recovery from antibody-antigen complexes of purified C1 molecules has been used in our laboratory to quantitate cell surface antigens. With this test we have been able to demonstrate tumor-specific antibody in sera of rabbits injected with diethylnitrosamine (DEN) induced tumors of guinea pigs. We studied two transplantable tumor lines and showed that each cell line contains three types of antigens: antigens common with normal guinea pig tissue cells; embryonic antigens present on both cell lines; and antigens specific for each of the tumor lines. At least 10^4 tumor-specific antibody binding sites per cell have been shown to be present on tumor cells. The availability of an in vitro humoral test for tumor antigens makes monitoring antigen purification processes much easier and makes quantitative studies on the nature, distribution, and significance of this antigen possible.

We are also continuing to study the mechanism of killing of nucleated cells (tumor and normal) by antibody and complement. We are studying the cytotoxic and complement fixing effects of antibodies directed against normal cell surface antigens and against tumor-specific cell surface antigens. One of the most striking findings to date has been the observation that of two different lines of guinea pig tumor cells (both sensitized with IgM antibody of the same specificity) one is lysable with complement, the other not. Yet when the cells are sensitized with the respective tumor-specific antibodies, both are lysable by complement. Thus susceptibility or resistance to lysis by complement may not be an absolute property of a given cell type but may be the consequence of the localization of the immunoglobulin in relation to the cell surface receptor for complement action.

Tumor-specific antibodies were also found in guinea pigs that received injections of tumor cells and subsequently were treated with BCG. This is a first demonstration of the presence of antitumor antibodies in guinea pigs against syngeneic tumor cells when the tumor was induced with DEN. We are investigating the specificity, nature and relationship of this antibody to tumor immunity in guinea pigs.

Molecular Separations Unit

Members of the Molecular Separations Unit (proposed Tumor Antigen Section) are pursuing a program that includes isolation and characterization of tumor-specific antigens, basic research on other cell membrane antigens and studies of immunological responses to cell surface antigens.

Last year we solubilized tumor-specific antigens from two lines of guinea pig chemically induced hepatomas. Detection of antigen was based on capacity of extracts to elicit delayed hypersensitivity responses in specifically immunized guinea pigs. Since then we have shown that antigen extracts purified by ammonium sulfate precipitation are reactive in two different test of cellular immunity: addition of antigen to suspensions of lymphocytes from

guinea pigs immunized to the specific tumor line causes blastogenesis of the cells as measured by increased incorporation of thymidine compared to controls. The antigen also causes elaboration of macrophage migration inhibitory factor, which can be measured as inhibited migration of normal guinea pig macrophages. In both of these *in vitro* tests, the stimulating activity of the antigen is specific for cells immunized to the tumor line from which the antigen was extracted.

It is of both practical and theoretical interest to determine whether immunization with antigen extracts protects animals against tumor challenge. This has been accomplished with extracts from both line 1 and line 10 tumors. Injection of antigen-Freund's adjuvant emulsions, followed by intradermal boosts of antigen in saline, is more effective than antigen without Freund's adjuvant.

For further purification of antigen it would be an advantage to have specific antibody against the antigen. By immunization of rabbits with whole tumor cells and appropriate absorptions of the resulting antiserum, we have obtained reagents which are specific for tumor antigen of both line 1 and line 10 cells. The specific antibody has been detected by the C1 fixation and transfer test and also by fluorescence on whole tumor cells, with a fluorescein-tagged anti-rabbit serum. Preliminary data indicate that both tests can be used for detection of soluble antigen.

Experience with the guinea pig tumor system is leading us into a major attempt to do similar studies on human solid tumors. We obtain tumor immediately after excision, make single cell suspensions and determine whether whole tumor cells or extracts of these cells induce blastogenesis of the patient's peripheral blood leukocytes in culture. Data are too preliminary for review here. However, establishment of a smooth working relationship with several surgical services at the National Naval Medical Center means that the Biology Branch now has a solid base for continuing clinical research.

We have continued basic studies on cell membrane function. Extraction of rat skeletal muscle under mild conditions yields a protein which regulated calcium entry into the cell. One of the proteins in the extract is immunochemically related to a plasma protein (called cardioglobulin) previously shown to mediate calcium transport across cell surfaces. It is of interest that by immunodiffusion there is a line of identity between the rat skeletal muscle extract, rat serum and human serum. Isolation of the component from rat and human serum is in progress.

Two studies on factors affecting immune response were pursued this year. One was the effect of complete Freund's adjuvant on the immune response. It was found that injection of guinea pigs with Freund's adjuvant impaired the capacity of the animal to reject a tumor challenge. Freund's adjuvant had little or no effect on an animal *previously* immunized to tumor. Thus the adjuvant impairs primary immune response and its action can be circumvented by systemic passive transfer of cells from an immune donor.

The other study relates to a control point in the action of complement. There is a serum protein called C3 inactivator which can abolish the biological activity of C3 once it has become activated. We have shown that C3ina attacks cell bound activated C3 only in certain instances, depending on the nature of antigen-antibody system on the cell surface. This study relates to our interest in the chemical basis of differences in susceptibility of cells to complement-mediated cytotoxicity.

Serial No. NCI-4670
1. Biology Branch, OASDC, Etiology
2. Office of the Chief, Molecular Separations Unit, and Cellular Immunity Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: In Vitro Assays of Specific Tumor Immunity

Previous Serial Number: None

Principal Investigators: Berton Zbar, M.D., Edward J. Leonard, M.D. and Herbert J. Rapp, Sc.D.

Other Investigators: Bruce H. Littman, M.D., Gerald L. Bartlett, M.D. and David A. Boetcher, M.D.

Cooperating Units: For supply of Mycobacterium bovis strain BCG: Trudeau Institute, Saranac Lake, New York. For studies of patients with cancer: Dr. Feller, Georgetown University, School of Medicine and Dr. Cole, Bethesda Naval Hospital. For preparation of soluble tumor antigen and for macrophage migration assay: Dr. Meltzer, Viral Leukemia and Lymphoma Branch, NCI. For macrophage migration Assay: Dr. Winthrop Churchill, Peter B. Brigham Hospital, Boston, Massachusetts.

Man Years:

Total:	1.6
Professional:	1.3
Other:	0.3

Project Description

Objectives

The primary objective of this project is the development of in vitro tests for measurement of tumor immunity in tumor immune and tumor bearing animals including man. Tumor antigens have been detected in this laboratory by in vivo tests. These tests involve the use of large numbers of animals and are not readily applicable to humans. On the other hand, in vitro tests use small numbers of animals, are applicable to humans and can measure levels of immunity.

Methods Employed:

Host-Tumor Systems: Two antigenically distinct ascites variants of diethylnitrosamine induced hepatomas were studied. Tests were performed using guinea pigs with progressively growing intradermal tumors and with immune guinea pigs. In mice, tests were performed with several antigenically distinct methylcholanthrene induced sarcomas. Patients with cancer were evaluated.

Tests

Lymphocyte transformation: Lymphocytes respond specifically to antigens by blast formation and cell proliferation. This proliferative response can be measured by incorporation of H^3-thymidine into lymphocyte DNA.

Inhibition of macrophage migration: Lymphocytes respond specifically to antigens to produce a factor which prevents the migration of macrophages from a capillary tube. The area of macrophage migration is measured. The degree of inhibition of migration correlates with the degree of immunity.

Antigens for In Vitro Tests: Soluble tumor antigens were prepared by extraction of freeze-dried tumor tissue with 3M KCL. In some tests, intact nondividing tumor cells were used.

Major Findings:

Studies with Guinea Pigs

1. Specific tumor immunity was detected in immunized guinea pigs by lymphocyte transformation and inhibition of macrophage migration.

2. A dose response was present to soluble tumor antigens.

3. Mitomycin-C treated tumor cells stimulated lymphocytes obtained from specifically immunized donors to transform. This proliferative response correlated well with response to soluble tumor antigens.

4. Tumor bearing animals had tumor immunity detectable in vitro.

5. Tumor immunity in tumor bearing animals was less than in immunized animals.

6. Lymphocytes obtained from immunized animals produced migration inhibitory factor in response to tumor antigens

Studies with Mice

1. The conditions required for lymphocyte transformation to purified protein derivative of tuberculin and to phytohemaglutinin were determined.

Studies with Humans

1. Mitomycin-C treated tumor cells stimulated transformation of autochthonous lymphocytes in 2 of 5 patients. Soluble tumor extracts did not stimulate lymphocyte transformation.

Significance to Biomedical Research and the Program of the Institute

The availibility of reliable simple in vitro tests of tumor immunity will permit a complete description of the tumor-specific immune response in the patient with cancer. The influence of chemotherapy, surgery and immunotherapy on this tumor specific immune response can be measured. Such tests may provide diagnostic as well as prognostic information. The factors responsible for progressive tumor growth or regression may be identified.

Proposed Course of Project:

Refinement of all in vitro tests will be done. The effects of immunotherapy and chemotherapy on tumor immunity will be measured. The problem of tumor immunity existing with a growing tumor will be investigated. The effect of serum factors from tumor bearing animals will be investigated in vitro. Development of other in vitro tests which measure other links in the immune response is in progress. A cell mediated cytotoxicity assay is to be devised.

Publications

Churchill, W. H., Zbar, B., Belli, J. A., and David, J. R.: Detection of cellular immunity to tumor antigens of a guinea pig hepatoma by inhibition of macrophage migration. J. Nat. Cancer Inst. 48: 541-549, 1972.

Serial No. NCI-4664
1. Biology Branch, OASDC, Etiology
2. Cellular Immunity Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Immunologic Approaches to the Prevention and Treatment of Cancer

Previous Serial Number: Same

Principal Investigators: Berton Zbar, M.D., and Herbert J. Rapp, Sc.D.

Other Investigators: Tomiko Tanaka, Ph.D., Gerald L. Bartlett, M.D., and Bruce Littman, M.D.

Cooperating Units: For supply of Mycobacterium bovis strain BCG: Trudeau Institute, Saranac Lake, New York. For studies of the histopathology of BCG-mediated tumor regression: Dr. M. Hanna, Jr., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. For studies with nonliving mycobacterial preparations: Dr. Edgar Ribi, Rocky Mountain Laboratory, Hamilton, Montana.

Man Years:

Total:	3.2
Professional:	2.2
Other:	1.0

Project Description

Objectives:

The primary objective of this project is to establish conditions and methods suited to the prevention and treatment of cancer. This objective is based on the finding that tumors contain unique antigens capable of eliciting the formation of tumor specific humoral antibodies and of causing the appearance of cells which react specifically with tumor antigens. Transplantable syngeneic guinea pig tumors will be used to investigate conditions for effective immunotherapy.

Methods Employed:

Tumors. Malignant hepatomas have been induced in strain-2 guinea pigs by feeding them diethylnitrosamine in their drinking water. Transplantable ascites tumors have been derived from primary hepatomas. Two of these ascites variants are being maintained by periodic intraperitoneal trans-

plantation. A strain-2 guinea pig leukemia is being maintained by periodic intradermal transplantation. Portions of these tumors are periodically frozen and stored in liquid nitrogen. The work in this report was carried out with cells of a transplantable hepatoma (line 1C) which when injected into the skin produces a progressively growing papule and regional lymph node metastases within a few days.

Tumor suppressive properties of mycobacterial preparations. In one assay, tumor cells and mycobacterial antigens were mixed in vitro and inoculated intradermally into strain-2 guinea pigs. The size of the inflammatory reaction and/or tumor was measured twice a week. In a second assay, tumor cells were injected intradermally into unimmunized strain-2 guinea pigs. When established intradermal tumors and regional lymph node metastases were present (7 days after intradermal injection) mycobacterial antigens were injected into the tumor.

Nonliving mycobacterial preparations. Bacterial cell walls were prepared in the laboratory of Dr. Edgar Ribi. Freeze-dried cell walls were mixed with a small amount of mineral oil and this mixture was ground to a smooth paste. Saline containing tween 80 was added to the paste and grinding was continued until a well dispersed oil in water emulsion was obtained.

Histopathologic studies. Complete autopsies were performed on guinea pigs. Portions of tissue were fixed in both Bouvin's fluid and Tellyesniczky's fixative. Five micron sections of tissue were stained with hematoxylin and eosin, Ziehl-Neelsen stain for acid fast organisms, or Snook's reticular stain.

Tumor-specific vaccines. The efficacy of vaccines containing mycobacterial antigens and tumor cells was studied in a model characterized by simultaneous contralateral injection of vaccine and challenge (living tumor cells) preparations.

Major Findings:

Nonliving Mycobacterial Preparations

1. The growth of syngeneic guinea pig tumor transplants in skin was suppressed if the tumor cells were inoculated together with BCG cell walls attached to oil droplets.

2. There was no suppression of tumor growth if the tumor cells were given together with: a) BCG cell walls, b) oil droplets and c) oil droplets and BCG cell walls prepared in a way that prevented the attachment of the walls to the oil droplets.

3. Animals in which tumor was suppressed acquired systemic tumor immunity.

4. Injection of BCG cell walls attached to oil droplets into established intradermal tumors led to tumor regression and prevented the

development of lymph node metastases.

5. Injection of M. smegmatis cell walls attached to oil droplets into established intradermal tumors did not lead to tumor regression or prevent the development of lymph node metastases.

6. BCG cell walls treated with sodium hydroxide retained ability to suppress tumor growth.

7. A chloroform-methanol extract of BCG cell walls mixed with oil droplets failed to suppress tumor growth.

Histopathologic Studies of BCG Mediated Tumor Destruction

1. The major finding is the presence of a granulomatous reaction at the tumor site and the regional lymph node. Histocytes appear to be the major effector cells in the reaction.

2. A lymphoproliferative response of the regional node (in the absence of histocytosis) does not inhibit tumor growth.

3. Cellular reactions in the regional lymph nodes (draining tumors treated with vaccina virus or oxazolone) which were characteristic of the development of delayed-type hypersensitivity had no detrimental effects on the tumor and did not alter metastatic growth.

4. Turpentine induced inflammatory reaction at the tumor site did not suppress tumor growth.

5. Turpentine and oxazolone treatments enhanced tumor growth in the skin.

Tumor Specific Vaccines

1. Under the best conditions palpable malignant tumors ceased growth and regressed completely due to a systemic tumor specific immune response.

2. Effective vaccines contained living BCG and viable tumor cells.

3. Vaccines with irradiated (12,000 R) tumor cells were as effective as those with unirradiated cells. However, the latter were occasionally tumorigenic in animals with tumor at a distant site and were considered unacceptable in a therapeutic situation.

4. Use of repeated vaccine injections or a larger dose of tumor cells in the vaccine did not improve the therapeutic effect.

5. Vaccines which did not affect tumor growth were: tumor cells emulsified in complete Freund's adjuvant, irradiated tumor cells without BCG or antigenically distinct tumor mixed with BCG.

6. A vaccine containing tumor cells and killed Bordatella Pertussis organisms was not detectably immunogenic.

7. A vaccine containing tumor cells, BCG and Bordatella Pertussis organisms was less immunogenic than a vaccine containing BCG and tumor cells.

Treatment of Established Intradermal Tumors by Intralesional Injection of Living BCG.

1. Guinea pigs treated by intratumor injection of BCG develop systemic immunity; some animals reject distant artificial metastases.

2. X-irradiation of intradermal tumors on the 8th day after tumor inoculation promoted complete regression of the intradermal tumor, but did not impair growth of tumor cells present in the regional lymph node.

3. Excision of BCG infected tumors did not interfere with the therapeutic result and may afford a therapeutic advantage in animals with 12 day tumors.

4. Excision of the intradermal tumor combined with intradermal injection of BCG did not retard development of lymph node metastases.

5. Complications of intralesional injection of BCG were: a) local Gram negative infections and b) in one experiment 3 guinea pigs previously immunized to BCG died within 24 hours after intralesional injection of BCG. Deaths appeared to be related to systemic anaphylaxis.

6. Antilymphocyte serum treatment inhibited BCG mediated tumor destruction.

Effects of BCG on Tumor Growth in Muscle

1. Line 10 tumor cells grew well in muscle.

2. Whereas BCG and line 10 cells injected together intradermally always led to tumor cell rejection and specific line 10 immunity, intramuscular injection of BCG and tumor cells led to this result in 50% of the animals.

3. Unlike tumors in the skin, tumors in the muscle were not successfully treated by intralesional injection of BCG.

Significance to Biomedical Research and the Program of the Institute:

BCG preprations are potent inhibitors of tumor cell growth. Although this observation has been repeatedly made, it has been difficult to harness the therapeutic potential of BCG. The studies in this laboratory are aimed at determining effective ways for using mycobacterial preparations in man. Our studies have shown some of the requirements for effective BCG treatment and some limitations of this type of treatment. Of particular importance

is the demonstration that nonliving mycobacterial preparations are potent inhibitors of tumor growth. Such preparations avoid the risks inherent in the use of living bacteria in patients with cancer. This demonstration may lead to the identification of the molecules of mycobacteria essential for tumor inhibition.

Proposed Course of Project:

Emphasis will be placed on developing a chemically defined mycobacterial preparation capable of inhibiting tumor growth. Different bacteria, will be assayed for tumor inhibitory capacity. Assays will include 1) ability of bacterial preparations to cause regression of established intradermal tumors and 2) ability of bacterial preparation to augment immunogenicity of weakly antigenic tumors.

Histopathologic studies will be performed on 1) tumor killing mediated by nonliving mycobacterial preparations and 2) tumor killing mediated by tumor cell vaccines. The origin of the cells participating in BCG mediated tumor killing will be determined.

Attempts will be made to study the distribution of living BCG in man. These studies will be performed in collaboration with Dr. Brigid Leventhal, Leukemia Service, NCI.

Publications

Zbar, B., Rapp, H. J., and Ribi, E. E.: Tumor suppression by cell walls of Mycobacterium bovis (BCG) attached to oil droplets. A brief communication. J. Nat. Cancer Inst., in press.

Hanna, M. G., Jr., Zbar, B., and Rapp, H. J.: Histopathology of tumor regression following intralesional injection of Mycobacterium bovis (BCG). I. Tumor growth and metastases. J. Nat. Cancer Inst., in press.

Hanna, M. G., Jr., Zbar, B., and Rapp, H. J.: Histopathology of tumor regression following intralesional injection of Mycobacterium bovis (BCG). II. Comparative effects of vaccina virus, oxazolone and turpentine. J. Nat. Cancer Inst., in press.

Bartlett, G. L. and Zbar, B.: Tumor specific vaccine containing Mycobacterium bovis and tumor cells: safety and efficacy. J. Nat. Cancer Inst., in press.

Zbar, B., Bernstein, I. D., and Rapp, H. J.: Suppression of tumor growth at the site of infection with living Calmette-Guérin Bacillus. J. Nat. Cancer Inst. 46: 831-839, 1971.

Zbar, B., and Tanaka, T.: Immunotherapy of cancer: Regression of established tumors after intralesional injection of living Mycobacterium bovis (strain BCG). Science 172: 271-273, 1971.

Zbar, B., Bernstein, I. D., Bartlett, G. L., Hanna, M. G., Jr., and Rapp, H. J.: Immunotherapy of cancer: Regression of intradermal tumors and prevention of growth of lymph node metastases after intralesional injection of living Mycobacterium bovis (BCG), in press.

Serial No. NCI-4665
1. Biology Branch, OASDC, Etiology
2. Cellular Immunity Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mechanism of Delayed Hypersensitivity and Tumor Graft Rejection

Previous Serial Number: Same

Principal Investigators: Berton Zbar, M.D., and Herbert J. Rapp, Sc.D.

Other Investigators: Ed Baik Chung, M.D. and Tibor Borsos, Sc.D.

Cooperating Units: For supply of germ-free guinea pigs: Mr. Bohner, Laboratory Aids Branch, Division of Research Services.

Man Years:

Total:	0.75
Professional:	0.25
Other:	0.50

Project Description

Objectives:

The main objectives of this project are: 1) to define the mechanism of the immunological rejection of antigenic tumors, and 2) to ascertain possible immunologic defects in tumor-bearing hosts to explain the progressive growth of an antigenic tumor.

The long-range objective is to develop methods of eliminating immunological defects in host animals to allow for optimal immune response to the tumor.

Methods Employed:

Malignant hepatomas have been induced in strain-2 guinea pigs by feeding them diethylnitrosamine in their drinking water. Transplantable ascites and intramuscular variants of these tumors currently maintained in this laboratory are used.

Malignant fibrosarcomas have been induced in inbred mice by implanting pellets of methylcholanthrene subcutaneously. A transplantable sarcoma tumor number 1038, has been selected for intensive study.

Delayed hypersensitivity in mice was measured by the footpad test. The size of the foot was measured before and after injection of antigen into the footpad. Antigen preparations were injected into the footpads of specifically immunized and control animals. An increase in footpad thickness that was not due to trauma, or irritation of the antigen preparation was considered a positive test.

Depression of cell mediated immunity was evaluated in mice that received simultaneous contralateral injection of tumor cell and living BCG preparations. Footpad tests were performed with purified protein derivative (PPD) of tuberculin at varying times after tumor inoculation.

Germ-free guinea pigs were obtained by Cesearean section from term pregnant females. These Cesearean delivered guinea pigs were kept in sterile incubators.

Major Findings:

1. Germ-free guinea pigs injected with living BCG survived and developed delayed cutaneous hypersensitivity reactions to PPD.

2. In mice, the delayed hypersensitivity response to PPD in mice was depressed 2 weeks after tumor inoculation. The delayed hypersensitivity response to PPD was absent 4 weeks after tumor inoculation.

3. Mice with negative footpad tests to PPD had positive lymphocyte transformation tests to PPD.

Significance to Biomedical Research and the Program of the Institute:

Depression of cell mediated immunity in animals and patients with cancer have been repeatedly observed. The mouse system offers an opportunity for determining the cellular and molecular basis for this impairment.

Proposed Course of Project:

The depression of delayed sensitivity associated with a progressively growing mouse sarcoma will be studied in detail. Variables to be explored include: 1) comparison of the immuno-depressive effects of antigenic and nonantigenic sarcomas, 2) effects of x-irradiated tumor cells, 3) tests for depression of the humoral immune response, and 4) analysis of lymphocyte-macrophage function in tumor bearing animals.

Publications

Bernstein, I. D., Wepsic, H. T., Zbar, B., and Rapp, H. J.: Tumor Immunity: Impairment in tumor-bearing hosts. J. Nat. Cancer Inst. 48: 873-880, 1971.

Bernstein, I. D., Thor, D. E., Zbar, B., and Rapp, H. J.: Tumor Immunity: Suppression of tumor growth in vivo initiated by soluble products of specifically stimulated lymphocytes. Science 172: 729-731, 1971.

Bernstein, I. D., and Wright P.: In vitro detection of enhancing antibody by the macrophage migration test. Nature 231: 25-56, 1971.

Serial No. NCI-4666
1. Biology Branch, OASDC, Etiology
2. Cellular Immunity Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Tumor-Specific Immune Reactions in Mice

Previous Serial Number: Same

Principal Investigators: Herbert J. Rapp, Sc.D., and Berton Zbar, M.D.

Other Investigators: Gerald L. Bartlett, M.D., Ed Baik Chung, M.D., Ph.D., and Tomiko Tanaka, Ph.D.

Cooperating Units: For supply of mice and tumors: Institute for Cancer Research, Philadelphia, Pa. For supply of BCG: Trudeau Institute, Saranac Lake, New York. For radiation facilities, Radiation Branch, NCI. For preparation of soluble antigens: Dr. M. S. Meltzer, Leukemia and Lymphoma Branch, NCI.

Man Years:

Total:	1.8
Professional:	1.5
Other:	0.3

Project Description

Objectives:

A long-range goal of this project is to improve our understanding and control of the processes by which a cancerous individual, animal or human, may be able to protect himself from his tumor. An important feature of such processes is the ability of the individual to specifically interfere with tumor growth by means of immune mechanisms. Such tumor-specific immune reactions tend to be relatively weak, even in experimental circumstances. The immediate objectives of the project are, 1) to study tumor-specific immune reactions to ascertain those factors which impose limitations on the effectiveness of the immunity, 2) to study, both inductively and deductively, means of strengthening antitumor immune reactions, and 3) to develop methods for the study of tumor-specific immunity *in vivo* and *in vitro*.

One successful approach to augmenting tumor immunity in guinea pigs has been to immunize with mixtures of tumor and BCG (*Mycobacterium bovis*) rather than with tumor cells alone; the immunizing tumor inoculum failed to grow pro-

Soluble tumor-specific antigen is prepared from solid tumors by the method developed for guinea pig hepatomas (see Project NCI-4667). Minced or lyophyllized tumor is extracted for 20 hours in 3M KCL, dialyzed and concentrated. This extract is further fractionated by precipitation in saturated ammonium sulfate.

Major Findings:

Process Involved in Tumor Suppression at the Site of BCG Infection

1. When tumor cells were mixed with living BCG prior to injection into syngeneic animals, the following results were obtained:
   a. Growth of all tumors tested (4) was suppressed in normal mice, when compared to inocula of tumor cells without BCG.
   b. Tumor was suppressed in 100% of animals when inocula contained $5x10^6$ living BCG, but in only 50% of animals when inocula contained $5x10^5$ BCG.
   c. Tumor growth was suppressed when tumor-BCG mixture was injected into mice receiving a therapeutic dose of isonicotinic acid hydrazide.

2. Admixture of heat killed BCG (killing proven by lack of growth in culture) with tumor cells led to only partial suppression of tumor growth.

3. Inoculation of living BCG into established tumors (4 mm diameter) either failed to affect tumor growth or slightly decreased tumor growth; regressions were not observed.

Effects of BCG on Tumor-Specific Immunity

1. Using four syngeneic tumors of various antigenic and biologic characters,
   a. Injection of tumor cell-BCG mixture (from $5x10^3$ to $5x10^6$ organisms per inoculum) did not significantly augment resistance to subsequent tumor challenge.
   b. Intratumor injection of $1.5x10^7$ living BCG, with excision of residual tumor, did not produce greater resistance to challenge than did temporary tumor growth without BCG.

2. Using primary, methylocholanthrene-induced sarcomas, the rate of tumor recurrence or metastasis following surgical excision was not afffected by infiltration of the tumor bed with BCG at the time of surgery.

Effect of Tumor Growth on Immunity to BCG (Preliminary)

1. In animals immunized to BCG, delayed hypersensitivity to mycobacterial antigens (footpad swelling to PPD) was inversely related with the presence of tumor in the animals.

gressively when mixed with BCG and strong tumor immunity resulted. This project has adapted this technique to a mouse tumor system to facilitate certain manipulations of the immune response and to permit comparison of the response of several tumors.

The work was intended to determine what types of processes are required to prevent tumor growth at the site of a BCG injection, and how such an interaction affects the subsequent development of tumor immunity.

Nonspecific crippling of immune mechanisms has frequently been observed in the presence of advanced malignant disease. This problem has previously been studied in this laboratory in a guinea pig system; the study is progressing in a murine model, which seems to be more amenable to manipulation and study.

Methods Employed:

A variety of mouse sarcomas of the C_3H strain are available: four have been used thus far. Three were induced in subcutaneous tissue, by 3-methylcholanthrene; one developed, without carcinogen treatment, during prolonged culture of connective tissue in a diffusion chamber. One of the chemically induced tumors is highly antigenic; it grows with difficulty in normal syngeneic mice but grows readily in pre-irradiated mice. Another MCA sarcoma grows slowly and has been moderately antigenic in previous tests. The third chemically induced tumor grows very rapidly, metastasizes to regional lymph nodes, and has not effectively immunized syngeneic mice in previous tests. The "spontaneous" sarcoma has an average growth rate and has been very weakly antigenic. Portions of the tumors are frozen and stored in liquid nitrogen. They are maintained by serial, approximately biweekly transplantation in syngeneic mice which have been immunosuppressed by thymectomy and 450 r of x-irradiation.

The solid sarcomas are minced and digested with Pronase and DNAase. Appropriately diluted suspensions of the tumor cells are injected intradermally, either alone or after mixture and short (10-30 min) incubation with living BCG. In other cases, living BCG is infiltrated into tumors which have been growing in syngeneic mice for several days. Tumors are measured biweekly. After growth of primary tumor grafts, tumors are excised. Evidence of tumor immunity is obtained by comparing the growth of a second tumor inoculum in pretreated mice with growth of a similar inoculum in mice receiving control treatments. Evidence for delayed hypersensitivity to BCG is obtained by measuring the degree of swelling of the footpad 24 hours after injection of 10γ of PPD.

Living BCG organisms are killed *in vitro* by incubation at 65° C for 60 minutes. Isonicotinic acid hydrazide is administered in the drinking water to achieve an *in vivo* mycobacteriocidal effect.

2. In BCG-immune, tumor-bearing animals with diminished footpad response to PPD, splenic lymphocytes retained the potential to undergo specific transformation when exposed to PPD *in vitro*.

Soluble Tumor-Specific Antigens (Preliminary) (See also NCI-4667)

1. KCL tumor extracts have produced slight delayed hypersensitivity reactions (footpad swelling) in mice immunized to the tumor extracted.

2. KCL tumor extracts have produced transformation of lymphocytes from tumor-immune animals.

3. KCL tumor extracts have not been immunogenic, as assessed by tumor rejection.

Significance to Biomedical Research and the Program of the Institute:

These results suggest that many, perhaps all, tumors may be inhibited if living BCG is present at the tumor site. The strength of this nonspecific effect of anti-BCG immunity seems to be weaker in the murine system (4 tumors studied) than in the guinea pig system (1 tumor studied), as judged by the amount of tumor tissue which can be rejected by the process. The effectiveness of BCG in INH treated mice, and the partial effect of heat-killed BCG support the possibility that a non-infectious mycobacterial preparation may have tumor suppressive potential. As yet, there is no evidence for a consistent effect of BCG on the development of tumor specific immunity.

Depression of immunity in cancer patients may contribute to infectious complications, and may compromise immune tumor resistance. An understanding of the mechanisms involved in such suppression could lead to methods to reverse or compensate for tumor-associated immune defects.

Proposed Course of Project:

A non-infectious BCG preparation (BCG cell walls attached to oil droplets) will be studied for its ability to suppress tumor growth. Model systems are being developed for the use of immunotherapeutic procedures (specific and nonspecific) in the treatment of intra-pulmonary tumors. Studies will be continued in the augmentation of specific tumor immune reactions by viable or nonviable BCG preparations, or by other agents such as neuraminidase. Integral to this portion of the project will be the improvement of soluble tumor antigens for use as standard reagents, and the further development and use of *in vitro* correlates of delayed hypersensitivity (lymphocyte transformation, inhibition of macrophage migration, and cellular cytotoxicity) to determine the mechanisms involved in alteration of tumor immunity by the agents studied. Work is in progress to sensitize mouse lymphocytes *in vitro* to antigens of syngeneic tumors.

Publications

Bernstein, I., and Wright, P. W.: In vitro detection of enhancing antibody by the macrophage migration test. Nature New Bio. 231: 25-26, 1971.

Bartlett, G. L., Zbar, B., and Rapp, H. J.: Suppression of murine tumor growth by immune reaction to the bacillus calmette-guerin strain of Mycobacterium bovis. J. Nat. Cancer Inst. 48: 245-257, 1972.

Serial No. NCI-4629
1. Biology Branch, CASDC, Etiology
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Mechanism of Cell Transformation

Previous Serial Number: Same

Principal Investigator: Joseph A. DiPaolo, Ph.D.

Other Investigators: Nicolae C. Popescu, Ph.D.

Cooperating Units: None

Man Years:

Total:	3.8
Professional:	1.6
Other:	2.2

Project Description

Objectives:

The primary objective of this project is to establish conditions and methods for the quantitative study of chemical transformation. The immediate targets of study are:

1. Development of rapid assay systems for chemical carcinogens suitable for the screening of compounds and populations.

2. Determination of ways to increase the susceptibility of primary cell lines or cell strains to chemical transformation.

3. Development of assays to determine whether *in vitro* transformation is accompanied by the appearance of tumor specific antigens.

4. Determination of the role of non-oncogenic and oncogenic viruses in chemical transformation.

5. Determination of the ultrastructural changes of cells during the course of *in vitro* chemical transformation so that they may be contrasted with normal cells and with information pertaining to viral transformation.

Methods Employed:

All procedures performed are with the view of quantitating phenomena in vitro. Such procedures are required in order to determine whether or not the transformation observed is due to the direct or indirect effect of the carcinogen and in order to study the early events associated with in vitro transformation. Cells used come from freshly isolated cells from animals that as controls have many of the attributes of "normal" cells and from cell lines which are known to exhibit some of the properties associated with non-transformed cells. Discrete cells are grown in complete medium in the presence or absence of irradiated rat or hamster cells. The cells may be derived from whole animal embryos or may be from specific organs. The cells are exposed to chemical carcinogen either prior to or subsequent to seeding the cells in the petri dish.

Approximately one week subsequent to treatment the cells are examined under phase or under stained conditions for number of transformed colonies, toxicity, and spectrum of morphology of both the normal and transformed colonies. The frequency of transformation is expressed in a number of different ways. These take into consideration the observed rate of transformation on a per-cell basis or on a number of colonies obtained.

Major Findings:

In experiments with hamster embryo cells that are plated to form colonies, prior treatment with 7,8-benzoflavone or benz(a)anthracene prevented the toxicity ordinarily associated with the potent carcinogens benzo(a)pyrene or 3-methylcholanthrene results with β-naphthoflavone were more variable, since it was capable of inducing some toxicity. Transformation rate increased on both a cell and colony basis. Thus, these results present clear evidence that the toxic and transforming activity of carcinogenic polycyclic hydrocarbons are dissociable and that the two processes, as has been suggested, are different. These results do not negate previous reports of the relationship of cytotoxicity to concentration and potency of the carcinogen. Some transformed colonies may be sensitive to higher concentrations of the same carcinogen; thus ordinarily these colonies may be eliminated, while additional colonies become transformed. The presence of the protective agent during exposure to the carcinogen seems to increase the rate of metabolic conversions of formation of a theoretical carcinogenic polycyclic compound to its reactive carcinogenic form.

X-irradiation of hamster cells prior to exposure to chemical carcinogens has resulted in an enhancement of transformation. As a continuation of this study we have investigated further the time relationship between irradiation and carcinogen exposure. Frequency of transformation is proportional to concentration of carcinogen. The number of transformed colonies observed with benzo(a)pyrene at 6 and 12 hours with and without irradiation was unexpectedly large, relative to the total colonies counted. This indicated that although the plated cells have attached, they have not completely adapted to substrate and are either eliminated or probably

are physiologically more prone to transformation. The increased transformation frequency observed appears to be time related; maximum enhancement is found if carcinogen is added 48 hours subsequent to irradiating the cells. Subsequently, there is a rapid loss of sensitivity which indicated that irradiation does not simply select for pre-existing cells genetically sensitive to transformation. There may still be temporary physiological states which are radiation resistant and highly sensitive to transformation, though it seems unlikely that these conditions would persist for at least two days. It is more reasonable to conclude that the increased sensitivity to transformation is due to direct action of irradiation on the cells. The study on interaction of chemical and physical agents is extremely important to the study of the causes of carcinogenic affects on a cell. It is important to determine whether radiation causes a somatic mutation which expresses itself as a neoplastic transformation or whether radiation has an indirect effect which facilitates the process of carcinogenesis. Radiation, either by x-irradiation or ultraviolet light have in this system failed to produce recognizable transformation.

We have reported induction of transformation in Syrian hamster cells by carcinogenic chemicals of different classes. However, chemicals such as urethan and diethylnitrosamine, known to be potent carcinogens in vivo, produced no transformation when applied to cells plated to produce discrete colonies. To investigate possible in vivo activation and to minimize false negatives, we modified the bioassay system with intraperitoneal injections of the chemical (mg/100gm body wt.) into pregnant hamsters on day 12 of gestation and removal of fetuses on day 14 to provide time for completion of metabolic interactions. When the cells were prepared as usual and cloned from a secondary or tertiary culture on an irradiated feeder layer without additional chemical, transformation was extremely significant. In fact, the high percent of transformation may probably be due to amplification of the transformation due to subsequent in vitro growth. Transformed colonies resulted from injections of 7 carcinogens at concentrations nonlethal to fetuses: urethan(1 mg), diethylnitrosamine (0.2 mg.), ethylnitrosourea (0.5 mg.), 4 nitroquinoline-Noxide (1 mg.), aflatoxin (0.1 mg), benzo(a)pyrene (0.5 mg), 3-methylcholanthrene (1 mg), and 7-12 dimethylbenz(a)anthracene (1 mg). The cloning efficiency (8-12%) indicated no significant toxicity relative to untreated cultures. Examination of colonies obtained from later passages showed further increase in transformation by chemicals that require activation and by those that act directly on cells can be demonstrated in vitro by injection of chemicals in pregnant animals during specific gestation periods.

The demonstrable ability of certain metabolic inhibitors to block carcinogenesis in vivo suggests their usefulness for in vitro studies of cell-carcinogen interactions. The present invesitgation concerns the inhibition of DMBA-^{3}H binding by protein inhibitors in vitro to Syrian hamster embryo cells. In addition, quantitative radioautographic results of the effect of inhibitors on RNA synthesis are reported. The concentrations of chemicals were selected so they would have some significance to cell survival. Chloramphenical and cyclohexamide, partially inhibited the toxicity of

7,12-dimethylbenz(a)anthracene and the incorporation of the tritiated carcinogen. In addition, leucine and uridine incorporation was inhibited by the protein inhibitors by approximately 50%. The growth inhibition obtained with the combination of inhibitor and carcinogen were of the same magnitude as those obtained by the protein inhibitors alone. The question remains what is the relationship of the depression of toxic effect and the reduction of binding; is it due to inhibition of reactions involving the active metabolite of the carcinogen with the protein, or is it an indication that another cell component is actually involved.

Numerous reports have been published on the formation of sarcomas from cells cultured in vitro by a variety of methods; but the formation of carcinomas as a result of the inoculation of untreated cells which have been cultured in vitro is seldom reported. Epithelial cells, particularly if obtained from a differentiated tissue such as liver, would make possible significant comparisons between epithelial and fibroblast-like transformation. We reported that a rat cell line which probably consists of differentiated parenchymal cells is refractory to some liver carcinogens but is capable of producing tumors similar to Reuber hepatoma when the cells are injected into irradiated animals. Chromosomes were examined by the usual colcemide, hypotonic pretreatment for karyotype analysis. The absence of apparently normal diploid cells indicated that all cells examined had an abnormal chromosome constitution. Karyotypic analysis of cells in the 2S range indicated the presence of new markers which, for the most part, consisted of two large metacentrics and one minute chromosome. These cells have a number of biochemical and morphologic properties that the tumors induced are carcinomatous and related to liver. These results prove that although cell lines may have properties associated with normal cells caution must be taken before a cell line can be used for carcinogenesis studies. Proof must be obtained that the cell lines are not capable for producing tumors.

The initial alteration in the cancer cell continues to be a subject of controversy. Although most such concepts recognize altered genetic function in the cells of the fully developed cancer, there is no agreement as to whether the initial change is genetic or epigenetic, one change or many, reversible or irreversible.

We have reported the lack of common karyotypic changes as a result of transformation and stated that the alterations appeared to be at random. Now we are in the process of using more refined methods of chromosome identification. We are determining the banding pattern of each chromosome by means of quinacrine mustard fluorescence and the Giemsa banding technique. Applying a stain procedure which reveals constitutive heterochromatin and cytological preparation of cells it is possible to determine if any rearrangement of heterochromatin has taken place. Using 3T3 cells, we have established that the heterochromatin arrangement of 7,12-dimethylbenz(a)-anthracene transformed cells is familiar to that of the controlled line on the other hand SV40 transformed results in what appears to be a highly specific and new arrangement of heterochromatin in a specific chromosome.

It is planned to continue this study to include other types of carcinogens as well as other cells.

Because of the chemical reactivity of carcinogens with DNA there has been much interest in the possibility that chemical carcinogens are mutagenic and may hold their carcinogenic property to this phenomenon. In our study, we compared the transformation and mutagenicity of a series of aromatic amine derivitives under similar conditions. Mutagenicity was measured by products of 8-azaguanine resistant colonies in Chinese hamster cells. Transformation was done using secondary Syrian hamster embryo cells. Cytotoxicity, mutagenicity, and transformation frequency increased with concentration and degree of reactivity of the derivative FAA. Whereas, the target for mutagens is DNA, chemical carcinogens are activated and capable of binding to diverse sites of a cell, the critical one is unknown. To determine whether somatic mutations are responsible for transformation by a reactive carcinogen, DNA must be shown to be the site of action and it must be shown that both phenomena occurred at the same specific site. Model compounds used were N-2-fluorenylacetamide (FAA), its proximate carcinogen, N-hydroxy-FAA and N-acetoxy-FAA which mimics the properties of the ultimate form in FAA rat liver carcinogenesis. This last compound was shown to be highly active as a transforming agent.

Significance to Biomedical Research and the Program of the Institute:

The need to study chemical carcinogenesis *in vitro* is obvious when one realized that carcinogenesis is really a problem in cell ecology. Consequently, *in vitro* carcinogenesis provides an opportunity to study chemical-target cell interaction and chemical-nontarget cell problems such as other effects of the chemical on cells without the complications of *in vivo* problems. Therefore, *in vitro* carcinogenesis has wide applications in terms of studying compounds in man's environment for the sake of control as well as for determining how they alter the physiological process of cells.

Proposed Course of Project:

It is planned to further study the conditions which are responsible for or can alter *in vitro* transformation. These will include the use of x-rays, combinations of carcinogens of different classes, the combination of *in vivo*-*in vitro* systems, and the effect of stimulators and inhibitors of mixed function oxidases on toxicity and transformation. The mechanisms of early events of carcinogen-cell interaction will be studied. Investigation of different proximate carcinogens and metabolic inhibitors is planned. In addition, experiments are planned to test whether neoplastic transformation has occurred as a result of direct induction by chemicals, as the result of selection or of viral activation. The testing of the tumorigenicity of cells exposed for different time intervals will continue.

Publications

Serial No. NCI-4629

DiPaolo, J. A.: Chemical Carcinogenesis In Vitro. In Clark, R. L., Cumley, R. W., McCay, J., and Copeland, M. M. (Eds.): Oncology 1970: A. Cellular and Molecular Mechanisms of Carcinogenesis; B. Regulation of Gene Expression (Proceedings of the Tenth International Cancer Congress). Chicago, Illinois, Yearbook Medical Publishers, Inc., Vol. 1, 1972, pp. 274-279.

DiPaolo, J. A., Donovan, P. J. and Nelson, R. L.: In vitro transformation of hamster cells by polycyclic hydrocarbons: Factors influencing the number of cells transformed. Nature New Biol. 230: 240-242, 1971.

DiPaolo, J. A., Donovan, P. J. and Nelson, R. L.: X-irradiation enhancement of transformation by benzo(a)pyrene in hamster embryo cells. Proc. Nat. Acad. Sci. 68: 1734-1737, 1971.

DiPaolo, J. A., Donovan, P. J. and Nelson, R. L.: Transformation of hamster cells in vitro by polycyclic hydrocarbons without cytotoxicity. Proc. Nat. Acad. Sci. 68: 2958-2961, 1971.

DiPaolo, J. A., Nelson, R. L. and Donovan, P. J.: In vitro transformation of Syrian hamster embryo cells by chemical carcinogens of diverse classes. Nature 235: 278-280, 1972.

DiPaolo, J. A., Nelson, R. L. and Donovan, P. J.: Morphological, oncogenic, and karyological characteristics of Syrian hamster embryo cells transformed in vitro by carcinogenic polycyclic hydrocarbons. Cancer Res. 31: 1118-1127, 1971.

Huberman, E., Donovan, P. J. and DiPaolo, J. A.: Mutation and transformation of cultured mammalian cells by N-acetoxy-N-2-fluorenylacetemide. J. Nat. Cancer Inst. 48: 837-840, 1972.

Oshiro, Y., Gerschenson, L. E. and DiPaolo, J. A.: Carcinomas derived from rat liver cells transformed spontaneously in culture. Cancer Res. 32: 877-879, 1972.

Serial No. NCI-4672
1. Biology Branch, OASDC, Etiology
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Investigation of Membranes of Chemically Transformed Cells In Vitro.

Previous Serial Number: None

Principal Investigator: Yuki Oshiro, Ph.D.

Other Investigators: Joseph A. DiPaolo, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.3
Professional:	1.1
Other:	0.2

Project Description

Objectives:

The primary objective of this study is to determine the differences in molecular structure in the membranes of normal and chemically transformed cells. The unrestricted growth of transformed cells may be related to the structural alteration of their membranes because transformed cells usually lose "contact inhibition" of growth. Therefore, the immediate objective is to show the loss of "contact inhibition" of growth and the requirement for low serum for growth and to investigate the possibility of the requirement of different factor(s) in serum for growth of chemically transformed cells. The second objective is to determine whether there are differences in the function of nutrient transport through membrane. The functional differences, if any, reflect the structural differences of the membranes.

Methods Employed:

Balb/c-3T3 cells were obtained from Dr. G. Todaro, cloned and transformed in vitro with chemical carcinogens such as 7,12-dimethylbenz(a)anthracene by Drs. DiPaolo and Takano. The fractionation of serum and β-globulin by Sephadex column was performed at 4°. For the study of transport through membranes, radioactive glucose, 2-deoxyglucose uridine, thymidine, and leucine are used.

Major Findings:

The chemically transformed 3T3 cells have lost "contact inhibition" of growth. Thus, they grow to much higher cell density until either the nutrient is depleted or too acidic for growth, whereas growth density of normal 3T3 cells is serum dependent. The chemically transformed 3T3 cells require less serum for growth compared to normal 3T3 cells at 10% serum concentration chemically transformed 3T3 cells have shorter doubling time than normal 3T3 cells.

Significance to Biomedical Research and the Program of the Institute:

Various chemicals have been known to cause cancer in animals and transformation of cells in vitro. Most transformed cells lack regulatory mechanism of cell division of non-transformed cells. The unrestricted growth may be the specific result of structural alteration(s) of the membrane brought about by specific action of chemical carcinogens. Therefore, it is very important to demonstrate the changes which occur in the membrane when cells are transformed.

Proposed Course of Project:

Project will be terminated at end of calendar year.

Serial No. NCI-4673
1. Biology Branch, OASDC, Etiology
2. Cytogenetics and Cytology Sectio
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Neoplastic Transformation of Guinea Pig Cells by Chemical Carcinogens In Vitro

Previous Serial Number: None

Principal Investigator: Charles H. Evans, M.D., Ph.D.

Other Investigators: Joseph A. DiPaolo, Ph.D.

Cooperating Units: Cellular Immunity Section and Office of the Chief, Biology Branch, NCI

Man Years:

Total:	1.6
Professional:	1.1
Other:	0.5

Project Description

Objectives:

The primary objective of this project is to establish conditions and method for the quantitative study of in vitro chemical transformation of guinea pig cells. The investigation complements the previously developed and curr in vitro chemical carcinogenesis system established within the Cytogenetics and Cytology Section employing Syrian hamster embryo cells and the tumor immunology program utilizing in vivo chemical carcinogen induced tumors developed in strain-2 guinea pigs within the Cellular Immunity Section.

The specific objectives of the project are twofold:

1. The development of a rapid assay system for chemical carcinogens suitab for the screening of compounds and populations. As an inbred strain with a low incidence of spontaneous malignancy strain-2 guinea pigs offer a mammal system in addition to the Syrian hamster for the analysis of chemicals as potential carcinogens and investigation of the mechanisms of chemical carci ogenesis at the cellular level.

2. The development of assays to determine whether in vitro chemical trans- formation is accompanied by the appearance of tumor specific antigens.

The Biology Branch has extensive experience with tumor immunology in strain-2 guinea pigs. A variety of in vivo and in vitro immunological techniques are presently being utilized. The availability of these techniques and the syngeneic system offered by the inbred strain-2 guinea pig make this system a better choice for immunological study at this time than the Syrian hamster. The development of neoplastic cells transformed in vitro by chemical carcinogens will permit assessment of cell surface antigen alterations associated with chemical carcinogens as well as further understanding of in vivo tumor immunity in strain-2 guinea pigs as a model system for carcinogenesis and tumor immunology in general.

Methods Employed:

The quantitative in vitro chemical carcinogenesis system developed employing Syrian hamster embryo cells within the Cytogenetics and Cytology Section has been utilized throughout this study. Cells are freshly isolated from whole or specific organs of strain-2 guinea pig embryos or fetuses and are grown in the presence or absence of irradiated rat, hamster or strain-2 guinea pig feeder cell layers. The cells as a mass culture or as individual cells are exposed to chemical carcinogen subsequent to seeding the cells in the petri dish.

Approximately one week subsequent to treatment colonies are examined under phase contrast microscopy or light microscopy following fixation and straining of the cells for the number of transformed colonies, toxicity and spectrum of morphology of both the normal and transformed colonies.

Major Findings:

Studies during the first 8 months have established:

1. The successful mass cell culture of strain-2 guinea pig embryo and fetal cells in Dulbecco-Vogt and minimal essential medium as well as other media incorporating 10% fetal bovine serum.

2. The ability of primary and secondary mass culture cells to clone with a high degree of efficiency in a variety of media with and without feeder layers. Cloning efficiencies of untreated cells as high as 50% with a feeder layer and up to 30% in the absence of a feeder layer have been regularly observed.

3. The development of toxicity following the addition of chemical carcinogens in both mass and cloned cell cultures was similar to that observed in hamster cells exposed to the same chemicals. Carcinogens from several chemical classes have been employed including the polycyclic compounds benzo(a)pyrene, 3-methylcholanthrene and 7,12-dimethylbena(a)anthracene as well as the non-polycyclic chemicals such as N-acetoxy-N-2-flourenylacetamide and N-methyl-N'-nitro-N-nitrosoguanidine.

4. Morphological cellular changes of guinea pig cells exposed to chemi carcinogens and compatible with in vitro chemical neoplastic transform as observed in Syrian hamster embryo cells have been seen. Isolation of these cells has not been possible due presumably to toxicity from the air supply to the incubators in which the cells are grown. Installation of activated charcoal filters in the air supply line appears to have decreased if not eliminated the toxicity. Carcinogen treated guinea pig cells presently are being passed in culture to allow the emergence and isolation of in vitro neoplastic transformed clones of cells.

Significance to Biomedical Research and the Program of the Institute:

This project extends the current system of in vitro chemical carcinogene within the Cytogenetics and Cytology Section to an additional mammalian system. It also broadens the in vivo chemical carcinogenesis investigat within the Cellular Immunity Section to include in vitro chemical neopla transformation of strain-2 guinea pig cells. Development of this system will permit further opportunity to study chemical-target cell interactio without the complications inherent in in vivo analysis. In vitro chemic neoplastic transformation of strain-2 guinea pig cells will provide another assay system in addition to the Syrian hamster for screening of chemicals as potential carcinogens and broaden our understanding of che carcinogenesis at the cellular level through inspection of intracellul and cell surface alterations accompanying neoplastic transformation.

Proposed Course of Project:

Conditions and methods will continue to be refined toward attaining optimal development of in vitro chemical transformation of strain-2 guinea pig cells as a reproducible, quantitative and reliable assay for potential chemical carcinogens. Cell surface changes of isolated neoplastic clones will be studied as part of the investigation of the mechanism of chemical carcinogenesis at the cellular level. Specifica this project will examine cell surface antigens. Firstly, the degree of in vivo and in vitro immunogenicity of the transformed cells will be established. This will be accompanied by analysis of cell surface antigens including organ specific, Forrsman, fetal or embryonic and tumo speicfic antigens. Secondly, the examination will proceed with the extraction, isolation, purification and characterization of the cell surface antigens seeking to define their relationship, if any, to chemic carcinogenesis as well as to tumor immunity. The elucidation of these relationships will provide further understanding of the mechanisms of chemical carcinogenesis at the cellular level and ultimately at the mo complex levels of tumor establishment and immunity in the host animal.

Serial No. NCI-4636
1. Biology Branch, OASDC, Etiology
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Physico-Chemical Characteristics of Complement Components

Previous Serial Number: Same

Principal Investigator: Tibor Borsos, Sc.D.

Other Investigators: G. Michael Loos, Ph.D. and Herbert J. Rapp, Sc.D.

Cooperating Units: Johannes Gutenberg University, Mainz, Germany

Man Years:

Total:	0.9
Professional:	0.7
Other:	0.2

Project Description

Objectives:

To isolate chemically and functionally pure serum complement components and to characterize the physico-chemical properties and structure functional relationships of these biologically active molecules.

Methods Employed:

Complement components are purified by a number of techniques including gel filtration, preparative ultracentrifugation (including the B XIV zonal ultracentrifuge), ion exchange chromatography, and a new technique for the separation of molecules based on differences in isoelectric point. The effects on structure and function of isolated complement components of a number of physico-chemical parameters, such as changes in ionic strength, trace metal content, temperature and osmolarity are studied with immunochemical and physico-chemical techniques.

Major Findings:

We have continued to characterize the structure and function of the first component of complement (C1). We found an activation step in the C1 molecule that occurs after the binding of C1 and before the appearance of the active form of the molecule, $C\bar{1}$. Evidence for this internal activation step was obtained by the use of two different inhibitors of $C\bar{1}$: $C\bar{1}$-inactivator (I1) and a pentosan-poly-sulfo-ester (Sp54). Sp54 and I1 do not inhibit the uptake by antibody-antigen complexes of C1. Sp54 inhibits a population of bound C1 not inhibitable by I1. C1 taken up in the presence of Sp54 are not inhibitable either by I1 or by Sp54. C1 taken up in the presence of I1 are inhibitable by Sp54 but not by I1. Sp54 is capable of inhibiting the hemolytic action of EAC1q4 while I1 is not. Our findings suggest that the internal activation step involves the subcomponent C1q; this is the subcomponent that binds the molecule to the antibody-antigen complex.

We also found that C1, in addition to antibody-antigen complexes, can also be activated by two lipid solvents, chloroform and ether, and by trypsin. Activation of C1 by these chemicals yields a molecule that is indistinguishable from that activated by complement fixing antibody-antigen complexes.

In trying to understand the mechanism whereby complement components are activated, we have searched for probes that would reveal relationships between structure and function. One of the most intriguing findings was the observation that the action of the active $C\bar{1}$ molecule on C2 could be mimicked by trypsin. Once the fourth component was cell bound, binding of C2 in cytolytic form could be accomplished by replacing C1 with trypsin. This showed that the site of the C2 molecule susceptible to enzyme attack by C1 was also susceptible to the action of trypsin.

Significance to Biomedical Research and the Program of the Institute:

Basic studies of chemical and functional relationships of complement components are directed toward an understanding of the mechanism of complement fixation in general, and have potential application to studies of mechanisms of host resistance to infectious disease and tumors. Specifically it is necessary to determine the factors which inhibit or enhance the immunologic response to antigens. In the case of complement mediated cytotoxi

reactions, isolation of cytotoxic antibody, cell antigens and complement in chemically or functionally pure form permits study of controlled reactions in which an estimate of the importance of a variety of chemical and physical conditions can be made.

Proposed Course of Project:

Further examination of the functional and structural properties of guinea pig and human C1 and C2 are in progress.

Publications

Loos, G. M., Borsos, T., and Rapp, H. J.: Activation of the first component of complement. Evidence for an internal activation step. J. Immunol. 108: 683-688, 1972.

Sassano, F. G., Colten, H. R., Borsos, T., and Rapp, H. J.: Resolution of the first component of guinea pig complement into three subunits, C1q, C1r and C1s. Immunochemistry, in press.

Serial No. NCI-4638
1. Biology Branch, OASDC, Etiology
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Biosynthesis and Ontogeny of Serum Complement

Previous Serial Number: Same

Principal Investigator: Tibor Borsos, Sc.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0
Professional:	0
Other:	0

Project Description

Objectives:

To identify the site or sites of synthesis of individual complement components by means of isolated organ and tissue culture, and to determine the effect of a number of chemicals (e.g. antibiotics, antimetabolites, carcinogens, etc.) on the synthesis of complement components at various stages of embryonic and fetal development, and to identify the site or sites of complement synthesis in fetal tissue.

Methods Employed:

Guinea pig or human organs are isolated and maintained *in vitro* during the period of observation. De novo synthesis is confirmed by including ^{14}C-labelled amino acids in the incubation media and counting the ^{14}C-labelled complement component with standard counting equipment. In addition, localization of the cell types synthesizing complement *in vitro* are detected by a hemolytic assay performed in agarose gel.

Major Findings:

None

Significance to Biomedical Research and the Program of the Institute:

Studies on the synthesis of complement components by isolated tissues and cells, because of the extreme sensitivity of the assays, provide some insight into the early events in the biosynthesis of proteins. In addition, the techniques used in this investigation provide a sensitive, rapid and easily performed assay for the effect of chemical and physical factors, including carcinogens, antibodies, steroids, ionizing radiation, etc., on the synthesis of a protein.

Proposed Course of Project:

Project suspended due to lack of personnel.

Publications

Colten, H. R., and Wyatt, H. V.: Biosynthesis of serum complement. Int. Arch. Allergy Appl. Immunol., in press.

Wyatt, H. V., Colten, H. R., and Borsos, T.: Production of the second (C2) and fourth (C4) components of guinea pig complement by peritoneal cells. J. Immunol., in press.

Serial No. NCI-4639
1. Biology Branch, OASDC, Etiology
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mechanism of Complement Fixation and Action

Previous Serial Number: Same

Principal Investigator: Tibor Borsos, Sc.D.

Other Investigators: Herbert J. Rapp, Sc.D., Edward J. Leonard, M.D., and G. Michael Loos, Ph.D.

Cooperating Units: Johannes Gutenberg University, Mainz, Germany

Man Years:

Total:	1.7
Professional:	1.3
Other:	0.4

Project Description

Objectives:

To apply rational complement fixation tests based on the fixation and transfer of the first component of complement to the analysis of antigen-antibody reactions, in particular in the search for cancer specific antigens.

Methods Employed:

The model for studying cytotoxic reactions mediated by antibody and complement consists of sheep erythrocytes, hemolytic antibody and guinea pig complement. Purification procedures for antibodies and the complement components include: preparative (large scale) gel filtration, ion exchange chromatography and preparative free electrophoresis. Other techniques used include precipitin and immunoelectrophoretic analysis, analytical, zonal and preparative ultracentrifugation and other immuno- and physico-chemical methods.

Major Findings:

The ultimate goal of this work is the development of immunological tools that permit exploration of (1) immunological approaches to cancer diagnosis and (2) possible host immune defense mechanisms against cancer. During the last year we have developed complement fixing rabbit antisera to tumor specific antigen of guinea pig tumor cells. The cell surface antibody-antigen reaction is measured with the C1 fixation and transfer test (C1FT test). The production of xenogeneic antitumor antibodies is an unusual achievement and its success greatly depended on the C1FT test developed in our laboratory. Tumor specificity was achieved by exhaustively absorbing antiserum made against one tumor line with cells of an unrelated guinea pig tumor. Criteria for tumor specificity was lack of reaction of absorbed antiserum with cells of the serum used for absorption, lack of reaction with other tumor cell lines and lack of reaction with normal tissues including the organ in which the tumor arose. We have shown that the tumor specific antiserum contains antibodies of the IgM and IgG classes; both of these are cytotoxic.

If the antisera are first absorbed with normal tissues cross-reacting and specific antitumor antibodies can be shown to remain. The cross-reacting activity was removed by absorbing the antisera with normal embryonic cells. We therefore concluded that tumor cells, in addition to normal cell antigen, contain a common embryonic antigen and are antigen specific for each tumor line.

Significance to Biomedical Research and the Program of the Institute:

Complement fixation is one of the most widely used diagnostic tools. The development and successful application of a radically new and very sensitive CF test, the C1FT test, opened up new possibilities in determining antibody-antigen reactions on cell surfaces.

Furthermore, cytotoxic reactions due to antibody and complement are prime examples of body defense mechanisms. We believe that fundamental research into the nature and mechanism of complement fixation and action will contribute greatly to the development of diagnostic tools and to the understanding of the mechanism of immune body defenses.

Proposed Course of Project:

This is a long-range project, and we expect little change in the scope of the work during the next few years. The ultimate goals of these projects are the development of better diagnostic tools based on fixation of complement and the elucidation of molecular events associated with the action of complement and antibodies. It is hoped that as a result of our program of inquiry into the basic problem of the interaction of antibodies, antigens and components of complement, tools will be developed that are of practical significance in our search for cancer antigens.

Publications

Borsos, T.: Immunoglobulin classes and complement-fixing activity. In Amos, D. B. (Ed.): Progress in Immunology. New York, Academic Press, 1971, pp. 841-848.

Borsos, T., and Opferkuch, W.: Kinetics and assay of complement components: Clustering and hemolytic competence of $C\bar{4}\bar{2}$ complexes at a single site. Int. Arch. Allergy Appl. Immunol., in press.

Opferkuch, W., Rapp, H. J., Colten, H. R., and Borsos, T.: The first component of complement: Hemolytic assays with EA, EAC4 and with rat and guinea pig late acting components. Immunochemistry 8: 517-523, 1971.

Rice, F. A. H., Ciavarra, R., and Borsos, T.: Effect of Leucogenenol on formation of 19S and 7S hemolysin in normal and splenectomized rats. Proc. Soc. Exp. Med. Biol., in press.

Serial No. NCI-4662
1. Biology Branch, OASDC, Etiology
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Immune Cytolysis

Previous Serial Number: Same

Principal Investigator: Sarkis H. Ohanian, Ph.D.

Other Investigators: Tibor Borsos, Sc.D., Barbara D. Hooks, Ph.D. and Berton Zbar, M.D.

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.8
Other:	0.2

Project Description

Objectives:

A study of the molecular aspects of antibody-C lysis and antigen topography of nucleated cells.

Methods Employed:

Antibodies to normal and tumor antigens are detected and quantitated by immune cytolysis, colony inhibition, C1 fixation and transfer (C1FT) and by uptake of I^{125} labelled antibody.

Immunochemical methods including Sephadex and DEAE chromatography, electrophoresis, immunodiffusion and ultracentrifugation are employed to isolate antibody protein and complement components.

Major Findings:

The malignant guinea pig cells, L1, L10 and L_2C appear to express less Forssman antigen than their normal tissue counterparts. Both L1 and L10 appear to have the same amount of Forssman antigen. L10 sensitized with anti-Forssman antibody is resistant to lysis by complement while L1 and leukemia are not. By C1FT tests both L1 and L10 sensitized with anti-Forssman antibody have the same number of C1 fixing sites per cell. L10 cells are lysable, however, when sensitized with specific anti-L10 antibody.

Human C gives a higher degree of lysis than rabbit and guinea pig C when cells are sensitized with the appropriate antibody.

L1 and L10 tumor cells have been maintained in culture for approximately four months and are presently in their 15th subculture. The effect of growth media on optimum growth and antigen expression indicate medium RPMI 1640 and Eagles #2 are best for L1 and L10. The L_2C leukemia will not grow in any of the media tested.

Significance to Biomedical Research and the Program of the Institute:

The elucidation at the molecular level, of the effects of antibody and complement on both normal and malignant cells may be important in understanding control of cell growth by immunologic mechanisms.

Control of tumor cell growth *in vitro* makes it possible to study the action of specific and nonspecific agents on the cells at different stages of their growth cycle. The knowledge obtained from *in vitro* experiments will be very helpful when applied to the study of more complicated *in vivo* conditions.

Proposed Course of Project:

More extensive work will be carried out on the effect of antibody and complement on lysis of nucleated cells. This will include the fate of cell bound antibody, the class of antibody involved and binding of the complement components to sensitized cells.

Attempts will be made to develop synchronous cultures of L1 and L10 in order to determine whether changes in susceptibility to lysis occur during various stages of the growth cycle. Also tests

for changes in surface antigen expression will be carried out with cells taken from different stages of growth.

Publications

Baker, A. R., Borsos, T., and Colten, H. R.: Cytotoxic action of antiserum and complement: Quantitation with a colony inhibition method. Immunology 21: 33-43, 1971.

Serial No. NCI-4669
1. Biology Branch, OASDC, Etiology
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Separation of L-Asparaginase from Complement Fixing Antigenic Impurities

Previous Serial Number: None

Principal Investigator: Tibor Borsos, Sc.D.

Other Investigators: G. Michael Loos, Ph.D. and Sidney Shifrin, Ph.D.

Cooperating Units: Johannes Gutenberg University, Mainz, Germany and the Macromolecular Biology Section, LCBGY

Man Years:

Total:	0.4
Professional:	0.3
Other:	0.1

Project Description

Objectives:

To separate L-asparaginase (E. coli) from contaminating bacterial antigenic material. To design methods for monitoring presence of biologically active impurities.

Methods Employed:

Standard biochemical and immunochemical methods for separation of macromolecules from complex mixtures are employed. They include gel filtration, ultracentrifugation, chromatography, complement fixation.

Major Findings:

In screening about 20 L-asparaginase preparations we found that most were capable of interacting with complement of selected human sera. The anticomplementary activity was shown to be distinct from L-asparaginase activity: on passing through a Sephadex G-200 column, all anticomplementary activity was in the void volume while all L-asparaginase activity was retarded.

Significance to Biomedical Research and the Program of the Institute:

It has been known that patients often react to L-asparaginase by exhibiting severe anaphylactic type reactions. If these undesirable reactions are primarily due to a bacterial, endotoxin type contaminant, its elimination from the enzyme preparations should relieve the patients from severe side effects and may permit the administration of more effective doses of the enzyme.

Proposed Course of Project:

Further purification and characterization of this undesirable contaminant is proceeding. Testing of anti-leukemic efficacy of the highly purified L-asparaginase and of anticomplementary contaminant is planned in animal models. If warranted, purified preparations will be also tested in patients.

Publications

Loos, M., and Borsos, T.: Inactivation of complement by L-asparaginase preparations: Lack of correlation with enzyme content. Nature, in press.

Serial No. NCI-4663

1. Biology Branch, OASDC, Etiology
2. Molecular Separations Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Interaction of Beta-1C Globulin with Antigen-Antibody Systems

Previous Serial Number: Same

Principal Investigator: Edward J. Leonard, M.D.

Other Investigators: Tibor Borsos, Sc.D.

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0.2
Other:	0.1

Project Description

Objectives:

To measure C3 (beta-1C globulin) binding to cell surfaces as a result of interaction of antigen-antibody complexes with complement. This is being done for several purposes: (1) as a sensitive detection system for very low concentrations of antibody; (2) to obtain evidence for different types of C3 binding as reflected by different sensitivity to the action of C3 inactivator; and (3) to study the basis for the differences among cells in susceptibility to complement lysis.

Methods Employed:

Sheep red cells, appropriate antisera, lytic tests, immunoelectrophoresis and the C1 fixation and transfer test were used. A variety of solid phase C3 intermediates were made. For studies on C3 inactivator, we used both normal serum and serum from a patient deficient in C3 inactivator.

Major Findings:

The effect of C3 inactivator (C3ina) in human EDTA plasma on C3 antigen bound to various immune complexes was determined. C3ina greatly decreased the amount of C3 bound to human erythrocytes sensitized with IgM anti-I cold agglutinin, and also decreased erythrocyte-bound C3 of a patient with deficiency of C3 inactivator, but had little effect on C3 bound to human red cells sensitized with Donath-Landsteiner IgG cold agglutinin. C3ina did not alter the amount of human C3 antigen bound to sheep E, to pneumococcal polysaccharide-antibody precipitate and to Sepharose-2B beads. Although C3ina did not alter the amount of C3 antigen on sheep E, a change in the molecule must have occurred since immune adherence was abolished. These data along with evidence from the literature show that several stages of bound C3 inactivation can occur.

Significance to Biomedical Research and the Program of the Institute:

Since C3 is pivotal in many of the reactions due to complement (cytotoxic, chemotactic, immune adherence, phagocytosis) the finding of different forms of C3 on cells may elucidate the mechanism of inhibition and amplification of cell damage by complement.

Proposed Course of Project:

We plan to quantify C3 binding to both erythrocytes and nucleated cells in different antigen-antibody systems and to study the effect of C3 inactivator on the bound C3.

Publications

Borsos, T., and Leonard, E. J.: Detection of bound C3 by a new immunochemical method. J. Immunol. 107: 766-771, 1971.

Leonard, E. J., and Borsos, T.: Effect of C3 inactivator on bound C3 antigen. J. Immunol. 108: 776-781, 1972.

Serial No. NCI-4667
1. Biology Branch, OASDC, Etiology
2. Molecular Separations Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Isolation and Study of Tumor-Specific Antigens

Previous Serial Number: Same

Principal Investigator: Edward J. Leonard, M.D.

Other Investigators: Monte S. Meltzer, M.D., Tibor Borsos, Sc.D., David A. Boetcher, M.D., Herbert J. Rapp, Sc.D., and Joost Oppenheim, M.D.

Cooperating Units: Viral Leukemia and Lymphoma Branch, VO, ET, NCI, and Laboratory of Microbiology and Immunology, NIDR

Man Years:

Total:	1.5
Professional:	1.5
Other:	0.0

Project Description

Objectives:

To obtain tumor-specific antigens in soluble and partially purified form. To use these antigens in inducing, establishing and measuring tumor specific immunity in guinea pigs. Knowledge derived from the guinea pig model will be applied to the isolation, purification and characterization of human tumor-specific antigens. These antigens will be then used in diagnostic studies of human tumor epidemiology, in monitoring patient response to treatment and as an aid in treatment. Initial studies will be on the ascites cell form of diethylnitrosamine induced hepatoma of strain-2 guinea pigs.

Methods Employed:

Initial extraction: 3M KCl and other hypertonic salts. Further purification: ammonium sulfate precipitation, gel filtration, DEAE chromatography. Assay methods: skin testing; *in vitro* correlates of cellular immune responses: lymphocyte blastogenesis, inhibition of macrophage migration; immunization of guinea pigs with extracts and challenge of test animals with live tumor cells. Production and testing of heterologous antisera to tumor antigen: immunization of rabbits with whole tumor cells, absorption of sera with various cell suspensions, testing of absorbed sera by C1 fixation and transfer test and by immune fluorescence.

Major Findings:

1. Activity and specificity of soluble line-1 and line-10 antigen in *in vitro* tests of cellular immune response. Last year we showed that line-1 soluble antigen caused positive skin tests in guinea pigs immunized with line-1 tumor cells, but not in line-10 immune guinea pigs; and vice versa for line-10 antigen. This specificity extends to two *in vitro* correlates of cellular immunity, blastogenesis of lymphocytes and inhibition of macrophage migration. Activity of antigen in these tests occurs only with cells from animals immunized with tumor of the corresponding line.

2. Soluble antigen has been used in various immunization schedules and we have found that injection of antigen-Freund's adjuvant emulsion into guinea pig footpads, followed by intradermal boosts of antigen in saline results in accelerated rejection of a subsequent challenge of line-1 tumor cells. Protection against line-10 cells by immunization with line-10 soluble antigen has also been achieved.

3. Injection of complete Freund's adjuvant without antigen results in reduced capacity to reject a tumor challenge given three weeks later. This is due to impairment of the primary immune response and the effect is bypassed by systemic administration of peritoneal exudate cells from an immune donor. Freund's adjuvant has no significant action on the secondary response.

4. Antisera from rabbits immunized by subcutaneous injection of whole tumor cells contain antibody against tumor-specific antigen. This was shown by analysis of antisera absorbed exhaustively with tumor cells of another line: C1-fixing activity of the serum with

the specific tumor line leveled off on a plateau and did not drop on further absorptions by cells of a different line.

Significance to Biomedical Research and the Program of the Institute:

The stability of the isolated antigen suggests that it would be possible to obtain it in a highly purified form suitable for studies on its chemical nature and for induction of specific antibody. Purified antigen and monospecific antibody would provide opportunities to experiment with anti-tumor vaccines, to detect development of antigen in tissue culture carcinogenesis models and to study host responses against neoplastic cells.

Proposed Course of Project:

Now that we have specific heterologous antisera against tumor antigen, it will be possible to follow antigenic activity during purification with more precision than with the skin test. Therefore we plan further purification steps as well as studies on the problem of antigen activity loss during the initial extraction. Detection and extraction of human tumor antigen are part of this program. Exploratory studies under way include attempts to demonstrate reactivity between patients' peripheral leukocytes and suspensions of tumor cells, KCl extraction of tumor cell suspensions, and a search for antibody against tumor cells either on the cells or in the serum.

Publications

Meltzer, M. S., Leonard, E. J., Rapp, H. J., and Borsos, T.: Tumor-specific antigen solubilized by hypertonic potassium chloride. J. Nat. Cancer Inst. 47: 703-709, 1971.

Serial No. NCI-4668
1. Biology Branch, OASDC, Etiology
2. Molecular Separations Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Cardioglobulin

Previous Serial Number: Same

Principal Investigators: Edward J. Leonard, M.D. and Stephen Hajdu, M.D.

Other Investigators: Laura Stewart, Ph.D.

Cooperating Units: Laboratory of Kidney and Electrolyte Metabolism, NHLI

Man Years:

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description

Objectives:

To develop immunochemical assay methods for free and cell-bound cardioglobulin; to apply these methods to the isolation and purification of cardioglobulin components; and to look for cardioglobulin alterations in disease.

Methods Employed:

Isolated frog hearts were used for bioassay of rat and human cardioglobulin components. Immunochemical and immune fluorescence methods were used to identify cardioglobulin antigens in solution and on tissue sites. Standard techniques of protein fractionation were used to separate cardioglobulin components from other serum proteins.

Major Findings:

Rat cardioglobulin-C. Last year we reviewed evidence that an antiserum against a G-200 large molecular weight fraction of rat serum (R113) contained antibody to rat cardioglobulin-C and that a cardioglobulin-C precipitin line (line-3) could be identified. R113 was used as a reagent for assay of cardioglobulin-C protein by (1) formation of precipitin line-3 or (2) inhibition by cardioglobulin-C fractions of R113 antibody binding to BC frog hearts or to rat skeletal muscle sections. The R113 antibody is detected on tissue sections with a fluorescein-conjugated antibody; inhibition of R113 antibody binding by antigen is referred to as IFI (immune fluorescence inhibition).

By three different serum fractionation procedures, line-3 precipitin activity was found over a wide range: from the first through third peaks on G-200 Sephadex gel filtration; from conductivities of 2 through 10 on gradient elution of DEAE columns at pH 8.0; and in more than half of a 10-28% sucrose gradient ultracentrifuge run when other precipitin lines showed the usual restricted ranges. However IFI identified a protein which was restricted to the first peak on G-200 elution and to a conductivity of 7-9 on DEAE chromatography. These results suggest that there are two anti-rat antibodies against two antigens on BC heart or rat skeletal muscle (the two antigens may be on a single molecule or two separate molecules). G-200 first peak and DEAE conductivity 7-9 regions have both antigenic determinants. One of the two determinants cause precipitin line-3 and spreads widely in G-200, DEAE and ultracentrifugation, probably because it tends to bind to various serum proteins.

Cross-reactivity of R113 with human protein. When R113 is reacted in gel against whole human serum a precipitin line is formed which makes a line of identity with line-3. R113 binds not only to BC hearts and rat skeletal muscle but also to human skeletal muscle membrane. Absorption of R113 with human serum causes diminished antibody binding to BC hearts (i.e. to the heart-bound rat serum component). Thus R113 can detect a serum protein in both rat and human which has in common the capacity to bind to autologous tissue membranes.

Isolation of human cardioglobulin components by frog heart bioassay. We have worked out standards for the three human components: B-hearts, a C-fraction (0-15% sodium sulfate precipitate) and an A-fraction (supernatant from the C-fraction). With two of the three standards we can assay for activity of one component. A activity is eluted from G-200 at 50-60% of bed volume. However C activity appears to be split on G-200 into two components, one of which is eluted in a narrow range (albumin region) and the other of which is distributed so widely that it must be heterogeneous (similar to the rat serum precipitin line-3 component).

Significance to Biomedical Research and the Program of the Institute:

Although there is much speculation in scientific literature about the possible role of calcium as a regulator of cell functions, including secretory activity, motility and contact inhibition, there is practically no solid evidence. We have shown that cardioglobulin is a serum protein system that can affect transmembrane calcium movement in isolated frog hearts. Isolation and characterization of the components of this system and the development of antisera to the components should enable us to answer questions about its tissue localization and biological function.

Proposed Course of Project:

Further purification of cardioglobulin components, production of monospecific antisera to these components and use of the antisera for studies of free and tissue-bound cardioglobulin in human disease and experimentally induced cancer in rats.

Publications

Hajdu, S., Posner, C. J., and Leonard, E. J.: Isolation from rat diaphragm of a calcium-protein complex involved in excitation-contraction coupling. Circ. Res. 29: 358-366, 1971.

SUMMARY REPORT
CHEMISTRY BRANCH
July 1, 1971 - June 30, 1972

The goal of the Chemistry Branch is to understand the molecular basis for malignant transformation by carcinogenic agents and modifying factors of exogenous and endogenous origin and the interaction of these agents with living organisms at the molecular, cellular and host level. We seek to clarify the consequences of these interactions in terms of cell regulation and carcinogenesis.

Cancer is clearly a disease of genetic expression where phenotype is altered in a heritable manner. This alteration of gene expression can be due to either genetic or epigenetic events induced by either xenobiotic or endogenous factors which result in altered patterns of gene control. The central focus of the Branch is to understand how exogenous carcinogens and endogenous factors are activated or deactivated by enzymatic processes and how the active forms interact with the gene action system producing the modifications which represent transformation. The aim of our studies is to understand these processes with a view towards the modification, elimination, or prophylaxis of the carcinogenic response in the human population.

The Office of the Chief and the Molecular Carcinogenesis Section are studying the molecular basis for malignant transformation induced by chemical carcinogens. The main focus is to understand the enzymatic conversion of carcinogens to either detoxification forms or to active carcinogenic metabolites. During evolution, human and other organisms have been exposed to a variety of carcinogens and other foreign compounds and have developed metabolic systems for their elimination. Our studies and others have shown that these detoxification reactions are influenced by a variety of environmental factors, such as drug intake, pollutant level, as well as previous exposure to carcinogens. We find that these systems are primarily detoxification mechanisms but may also activate ingested procarcinogens to ultimate carcinogenic forms. These studies have been primarily concerned with polycyclic hydrocarbons, the carcinogenic agents universally present in combustion products, polluted air, smoke and industrial wastes. The enzymes metabolizing these agents have been studied extensively in respect to the regulation of their synthesis, degradation, and activity. Our studies have demonstrated the universality of the enzyme system in mammalian tissues including human tissue as well as in a variety of cells grown in culture. Current efforts are designed to study the enzyme in the human population determining the relationship of enzyme level to sex, genetic

character, ethnic background, nutritional state and age of the individual and eventually to carcinogen susceptibility. During the last year we have identified the enzyme in human tissue and progressed in modification of the assay for use on minute amounts of tissue. The enzyme system has been found inducible in human tissue by the addition of polycyclic hydrocarbons which indicates that human enzyme level is regulated by exogenous factors. In experimental studies in both cell culture and in mice, several potent inhibitors of the carcinogen-metabolizing enzyme system have been discovered. One inhibitor is a flavone derivative and two are derivatives of polycyclic hydrocarbons. These compounds markedly inhibit tumorigenesis produced by dimethylbenzanthracene in mouse skin and protect against the toxic effects of hydrocarbons. In contrast benzopyrene-induced tumorigenesis is enhanced by the flavone inhibitor. These results indicate that each polycyclic hydrocarbon has a unique pathway of metabolism. The present goal is to elaborate the complete pathway of polycyclic hydrocarbon metabolism to active or detoxification forms with the view toward the modification of these processes to reduce carcinogenicity of this class of ubiquitous carcinogens.

The regulation of the aryl hydrocarbon hydroxylase enzyme system is complex and appears to involve activation of specific genetic loci. These studies have been advanced by the development of cell lines derived from liver which are inducible for this enzyme and the construction of hybrid cells that have been formed from parent cell lines that do not contain the enzyme system with other parent cells that exhibit enzyme inducibility. The use of these liver cell lines and hybrid cells will help to clarify the genetic, and chromosomal factors, in the molecular biology of the regulation of this enzyme complex which is important to carcinogenesis. In other studies we find that the genetic activation involving RNA synthesis required for enzyme induction does not depend on the concomitant synthesis or transport of ribosomal RNA to the cytoplasm.

Another program has involved the testing of over 50 compounds for their ability to induce or inhibit the carcinogen metabolizing enzyme system. Some of the compounds showing activity are under test in animal tumorigenesis systems.

The Cell Growth Regulation Section seeks to understand the biochemical basis for malignancy using a variety of biochemical and biological techniques. One project is to understand the mechanism of messenger RNA translation by studying the various factors affecting translation with particular emphasis on the role of transfer RNA. Transfer RNA's are important in the regulation of protein synthesis and in some cell differentiation systems. The translation of normal messenger RNA's as well as those foreign to the

cell, such as oncogenic viral messenger RNA's, may be important in malignant transformation by either chemical or viral carcinogens. Studies are focused on determining the normal profiles of tRNA in different tissues and tumors, the possible interaction of carcinogens with tRNA and the metabolic consequences of these interactions in respect to transformation.

Another project utilizes oncogenic viruses which transform cells to the malignant state rapidly and efficiently. The initial genetic changes responsible for transformation occur within one hour after exposure of the cells to the virus, and obvious malignant changes are observed within 24 hours. Experiments using a compound which disrupts the mitotic process (vinblastine sulfate) demonstrated that cellular divisions were not a requirement for virus reproduction, disproving a widely-held view that virus cannot reproduce without an intervening cellular division. Transformation of cells by Rous sarcoma virus also may occur without cellular division since morphological changes characteristic of Rous transformed cells are found after infection even though cellular divisions are prevented. Also, increased hexose uptake and increased hyaluronic acid synthesis, biochemical changes often found in malignant cells, are found in infected cells which have never divided after exposure to virus. Studies which indicated that an intracellular viral DNA was made and functioned as a replicative form for the production of progeny viral RNA, led us to attempt the experimental induction of mutants of Rous sarcoma virus. These attempts have been successful and certain of the mutants found cause infected cells to behave as malignant cells at one temperature but behave similarly to normal cells at a higher temperature. Both morphological and biochemical parameters of malignancy change with the temperature. An analysis of the metabolic requirements for transformation indicated that DNA, RNA, and protein synthesis could be inhibited without observable effect, indicating that the temperature-sensitive molecule responsible for the morphological change probably was a protein. A major effort of the laboratory is now concerned with the search for an identification of the specific protein.

The Nucleic Acids Section is engaged in three general areas of research. One is the investigation of the early subcellular events that occur following the infection of a susceptible mammalian cell with an oncogenic RNA virus. Current evidence indicates that immediately upon infection, the RNA genome of these viruses is transcribed into DNA which later integrates with the genome of the host cell. Attempts are being made to localize the subcellular sites of viral-directed processes that occur following infection. Research of the past year indicates that the viral RNA genome is converted by "reverse transcription" into a DNA-RNA hybrid in the surface membrane of the host cell. The subcellular site of the second conversion into a double-stranded, wholly DNA genome is apparently

not in the surface membrane, but at a different locale within the cytoplasm. The exact site of the latter event remains to be elucidated. An understanding of the subcellular location of each of the steps leading from virus infection to malignant transformation would permit the exploration of potential drugs or immunologic prophylaxis of this transformation process.

A second area of activity involves the development and refinement of the techniques of gel electrophoresis for the purpose of identifying the chemical and structural properties of nucleic acids isolated from viruses, bacteria and higher organisms. Techniques used in the past for this purpose were relatively ineffective being laborious, expensive, and requiring large amounts of highly purified material. Studies in collaboration with the Protein Section have greatly extended the usefulness of polyacrylamide gel electrophoresis in analyzing the kinds and properties of nucleic acids found in different samples of biological material. This technique is relatively inexpensive, simple, rapid, and requires only very small amounts of material (frequently less than 1/100,000 of a gram), which does not always have to be of high purity. Recent studies have shown that this technique can determine whether a newly isolated, discovered, or synthesized nucleic acid is RNA or DNA, is single- or double-stranded, and its approximate molecular weight. In some instances, the method can determine whether the molecule is linear or circular, and its heterogeneity. With high molecular weight DNA's, the method is useful in determining polydispersity with respect to nucleotide composition. The development of this technique is of the utmost importance to the study of viral genomes (oncogenic and otherwise), in vivo nucleic acid metabolism in bacterial cells and the cells of higher organisms, and the nucleic acid products of in vitro replication and transcription systems. The technique represents an important advance in the methodology of cancer research and biomedical research in general. Its application has universal relevance to biological systems.

Research efforts are directed toward the development of methods for analyzing the mammalian (including human) cell processes required to repair damage to the genetic material. Damage inflicted upon genetic material (DNA), may lead to mutation resulting in cell death, aberrant behavior or possibly malignant transformation. That malignant transformation could be a result of unrepaired or improperly repaired DNA damage is suggested by the finding that patients with xeroderma pigmentosum (a disease characterized by a high frequency of skin tumors originating on areas exposed to light) have a much reduced ability to repair ultraviolet light induced damage to their DNA. However, there are a few patients with this disease which do not exhibit defective DNA repair mechanisms. The possibility still exists, however,

that the current methods of assay for repair deficiency fail to include all aspects of the repair process and that these patients have a defect in DNA repair at a site other than that examined. Current studies aim to improve the assay for the overall effectiveness of DNA repair mechanisms in various cell types. If successful, it might be possible to study the relationship between the ability to repair DNA damage and susceptibility to malignant transformation. It would also then be possible to determine the effect of chemical, physical, or viral carcinogens on the repair mechanisms as well as on their direct effect on DNA.

The Protein Section has been concerned with the development of better methods for the separation of ribonucleic acids and proteins. The development of new knowledge on biological processes depends on both creative insight and the methodology which enables the investigator to experimentally answer the questions posed. An important requirement for understanding the biochemical events of cell regulation and carcinogenesis is the understanding of the metabolism of macromolecules concerned in regulation. This requires an ability to separate them from one another, isolate them and characterize the nature of the molecules. The Protein Section has been largely concerned and in part collaboratively with the Nucleic Acids Section with the advancement of methodology for the isolation and characterization of RNA and protein on polyacrylamide gels. The advances made in this area have had significant impact in nucleic acids research. Most recently, we have been concerned with the possibility of separating DNA of different types by this method. Last year we reported the separation of mouse DNA by gel electrophoresis by DNA fractions. One DNA corresponded to the satellite DNA, a DNA known to be associated with the centromere of chromosomes, and the other was the main-band mouse chromosomal DNA. Subsequently, satellite DNA of crab was also successfully isolated. The satellite DNA's differed from the main-band DNA in a number of characteristics including base composition. In order to evaluate how the base composition of a DNA species may determine electrophoretic mobility, DNA was prepared from a variety of bacteria with DNA with a wide range of base composition. DNA's with a higher percentage of guanine and cytosine migrated more rapidly than DNA's with a lower percentage. We hypothesize that the chains of a double helical of DNA rich in G-C are more tightly held together than in DNA's relatively rich in adenine and thymine. High G-C DNA's thus present a narrower cross-section than DNA's high in A-T, and hence penetrate gels more readily. Presently our studies are being extended to DNA from human tumors. In an extension of these studies, DNA from human tumors are treated at elevated temperatures to dissociate the chains. We are investigating the hypothesis that those chains bound more tightly because of a high G-C content will denature less readily. The combined

methods of heat stability and electrophoretic mobility represent a new approach to the characterization of the DNA found in the nucleus of normal and malignant cells. This is of particular importance since often malignant cells contain abnormally high chromosome number and it is of great interest to compare the nature of their DNA compared with normal DNA. The technique also offers the opportunity of intimately examining the nature of binding of carcinogens to DNA. In particular it may be possible to determine unique DNA's or sequences within the DNA molecules that are uniquely susceptible to carcinogen binding.

Other studies are concerned with the extraction of RNA and its analysis by gel electrophoresis. Our results, in summary, have shown that salt and/or detergent concentration, temperature of extraction, and pH, all influence the kind and amount of rapidly-labeled RNA that is extracted. These comparisons indicate that there is a wide variation in results dependent on the procedure used. Since the rapidly labeled RNA is of key importance in cell regulation, it is especially important for the study of carcinogenesis that valid samples can be obtained for analysis. We are hopeful that the results of our studies will provide insight into the proper methods of preparation of RNA.

In collaboration with the Nucleic Acids Section, we studied the effect of methionine deprivation or ethionine treatment on RNA metabolism in HeLa cells. Ethionine is an unusual carcinogen which is an analog of the natural occurring amino acid methionine. We found that cells treated with ethionine, or deprived of methionine, immediately cease to manufacture ribosomal RNA, although the other forms of nuclear RNA were not affected. Deprivation of methionine during a three or four hour period had no effect on the stability of ribosomal RNA suggesting that methionine deficiency affects either the synthesis or the stability of newly-formed ribosomal RNA. This new information may relate to the carcinogenic activity of ethionine.

Prolactin is a key factor in mammary gland regulation. Thus, certain mammary tumors fail to develop without the presence of prolactin and mammary tumors formed in the presence of adequate prolactin regress when prolactin is removed. Prolactin is an endogenous factor required for mammal lactation as well as abnormal tumor development. Last year we found that prolactin greatly stimulated the incorporation of precursors into RNA of mammary tissue explants, and increased the amounts of ribosomal RNA. Since prolactin is such a vital factor in mammary carcinogenesis a sensitive assay for this agent which is present in minute amounts would be necessary for progress in this area of carcinogenesis research. Towards this goal we have studied the proteins which are synthesized

in mammary explants when very low levels of prolactin are present. The major technique employed was the gel electrophoresis of proteins found in the tissue explant supernatant fluids. Most proteins normally found in mouse milk were present in very small quantities, but one particular protein was found in substantially increased amounts. These results are very promising in the potential development of a sensitive assay for prolactin.

The role of hydrocortisone in relation to prolactin activity was also investigated. We found that the pattern of RNA synthesis changed when hydrocortisone was present. Further, in the absence of hydrocortisone, prolactin-dependent casein production did not occur. Thus, the aspect of our research program is concerned with evaluating the hormonal factors relevant to mammary differentiation and carcinogenesis.

Studies with cyclic AMP have shown that phosphorylation and dephosphorylation of enzymes and other protein molecules is a key mechanism in the control of cellular metabolism. In respect to mammary carcinogenesis, milk proteins show a high degree of phosphorylation. A technique identifying phosphorylated proteins would find wide application in studies in the regulation of cellular metabolism and especially in malignant transformation. We have studied a stain which seems applicable to phosphorylated proteins. This stain affords a large increase in sensitivity for those proteins which contain phosphorus, increasing the level of detectability approximately 5-fold. We plan to look for such proteins in normal and virus-infected mammary cells as well as cells which have been transformed or altered by chemical carcinogens.

Several laboratories, including ours, reported populations of patients with leukemia in which the frequency of the haptoglobin gene number 1 was higher than expected on the basis of control populations. The reason for this is now known. Nonetheless, it appears that individuals homozygous for haptoglobin type 1 are at greater risk of development of leukemia than individuals heterozygous, or homozygous for gene 2. This inherited susceptibility may not be confined to leukemia, but may extend to other forms of cancer and other diseases. Epidemiological data suggests that persons with carcinoma of the pancreas might show such a preponderance of individuals with gene 1. To test this two contracts were let to provide data. On the basis of untreated data, a very significant excess of individuals of type 1-1 have been found in the population of carcinoma of the pancreas. There are two alleles of the gene 1 found normally, in various distributions, in all populations. We recently developed an improved methodology for determining this subtype and plan to apply this technique to all serums collected with genes type 1 or 2.

Serial No. NCI-4520
1. Chemistry Branch, OASDC, Etiology
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Biochemistry of Transformation of Cells by Avian Sarcoma Viruses

Previous Serial Number: NCI-4750

Principal Investigators: John P. Bader, Ph.D., and Michael Lew, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.5
Professional:	1.5
Other:	1.0

Project Description

Objectives:

To delineate the basic biochemical defect responsible for the change to malignancy in Rous Sarcoma virus-infected cells.

Methods Employed:

Cell culture, microscopic identification of cellular transformation, radioactive tracer techniques, isolation and purification of hyaluronic acid, polyacrylamide and agarose gel electrophoresis.

Major Findings:

Transformation of chick embryo cells by Rous Sarcoma virus (RSV) is accompanied by changes in hexose uptake and increased hyaluronic acid synthesis. We have concentrated on various aspects of hyaluronic acid on the possibility that this substance may be responsible for the malignant character of RSV-transformed cells. A rapid sensitive quantitative assay for hyaluronic acid was developed using electrophoresis in mixed gels of ployacrylamide

and agarose to separate the hyaluronate from other substances. Kinetics of synthesis in cell cultures has been analyzed using incorporation of radioactive glucosamine and qualitative as well as quantitative differences between RSV-transformed cells and normal cells have been established.

Mutants of RSV which transform cells at 36° but not at 41° were induced with 5-bromodeoxyuridine. The isolated virus mutants are capable of complete cycles of reproduction at 41°. Transformation of cells can be detected within 10 minutes after shifting cultures from 41° to 36°, and is virtually complete within 6 hours. Increased hexose uptake and increased hyaluronic acid synthesis are also found after shifting to the lower temperature. Inhibition of DNA synthesis, RNA synthesis, or protein synthesis has little effect on the morphological change, but prevents the increase in hexose uptake and hyaluronate synthesis. These results suggest that changes in hexose and hyaluronate metabolism, even though possibly important to the malignant process, are secondary to a more basic change reflected in altered morphology.

Significance to Biomedical Research and the Program of the Institute:

These findings encourage us to think that the delineation of the specific molecule responsible for malignancy induced by Rous Sarcoma virus is within sight. Such a determination would be a major step in defining the nature of cancer, and may be useful in devising programs for the control of cancer in man.

Proposed Course of Project:

To search for temperature sensitive molecules in cells infected with the mutant virus in an attempt to define the specific biochemical defect responsible for malignancy in this system. Also, to extend our studies on hyaluronate to determine what role this substance plays in the malignancy of RSV-transformed cells.

Publications

1. Bader, J. P. and Brown, N. R.: Induction of mutations in an RNA tumor virus using a DNA precursor analogue. Nature New Biology, 234: 11-12, 1971.

2. Bader, J. P., Ray, D. A. and Steck, T. L.: Electrophoretic determinations of hyaluronate produced by cells in culture. Biochim. Biophys. Acta (in press)

3. Oten, J., Bader, J., and Johnson, G. S., Pastan, I.: A mutation in a Rous Sarcoma virus gene which controls cyclic AMP levels and transformation. J. Biol. Chem. 247: 1632-1633, 1972

Serial No. NCI-4748
1. Chemistry Branch, OASDC, Etiology
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Synthesis and Characterization of The RNA of RNA-containing Tumor Viruses

Previous Serial Number: Same

Principal Investigators: Yoshiyuki Kitano, Ph.D. and John P. Bader, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.3
Professional:	1.1
Other:	0.2

Project Description

Objectives:

To determine the molecular structure of the RNA of RNA-containing tumor viruses and to elucidate the intracellular site and mechanism of synthesis of viral RNA.

Methods Employed:

Cell culture, radioactive tracer techniques, density-equilibrium and sedimentation rate centrifugations, isolation and purification of nucleic acids, polyacrylamide gel electrophoresis, electron microscopy.

Major Findings:

The conformation of the RNA of RNA tumor viruses has been under investigation for several years. Partial characterization of the isolated viral RNA has been made, and a core particle devoid of virion envelope has been analyzed. During this past year it

was found that treatment of intact virions with diethyl pyrocarbonate, a reactive compound used in the chemical inactivation of enzymes, followed by detergent treatment resulted in nucleoprotein particles not previously described. The biophysical homogeneity suggests that the particles were a product of the association of internal virion protein with the viral RNA.

Significance to Biomedical Research and the Program of the Institute:

It may be possible, by similar treatments, to force associations of viral protein and RNA within the infected cell. Analyses of such complexes may help describe intracellular aspects of virus reproduction, and the functioning of the viral RNA which is responsible for tumorigenesis by the RNA tumor viruses.

Proposed Course of Project:

To define the site of viral RNA synthesis, to identify factors involved in viral RNA synthesis, to follow the newly synthesized molecule into cellular organelles, and ultimately to describe the products of the viral genes.

Publications

1. Bader, J. P.: The RNA of RNA-containing tumor viruses. In Dutcher, R. M. (Ed.): Comparative Leukemia Research, New York, Karger, 1970, pp 140-143.

Serial No. NCI-4759
1. Chemistry Branch, OASDC, Etiology
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Nonsense Codon Recognition in Mammalian Tissues

Previous Serial Number: NCI-4759

Principal Investigators: Dolph Hatfield, Franklin Portugal, Ph.D., and Ione Polacow, Ph.D.

Other Investigators: None

Cooperating Units: Viral Carcinogenesis Branch, NCI

Man Years:

Total:	3.2
Professional:	2.2
Other:	1.0

Project Description

Objectives:

To detect and analyze species of tRNA in mammalian liver which recognize the nonsense codons UGA, UAG & UAA. To determine if tRNA species which recognize nonsense codons and if other mammalian tRNAs are capable of suppressing nonsense mutations in protein biosynthesis.

Methods Employed:

Assay of bovine liver tRNA fractionated by reverse phase chromatography (Kelmers et al, J. Biol. Chem. 240: 3979-3983, 1965) for recognition of labeled nonsense codons by the procedure of Nirenberg & Leder (Science 145: 1399-1407, 1964). Further purification of tRNA species which recognize nonsense codons by a combination of reverse phase chromatographic and B-D cellulose columns. Amino acylation of fractionated mammalian tRNA with E. coli amino acyl-tRNA synthetases to devise an E. coli protein synthesis system.

Major Findings:

Seryl-$tRNA_{UGA}$ (Proc. Nat. Acad. Sci. USA 67: 1200-1206, 1970 and Cancer Res. 31: 697-700, 1971) has been isolated in high purity. It has been resolved into two species (both recognize UGA) by chromatography on B-D cellulose. It recognizes specifically UGA at 0.02m Mg^{++} and with limiting levels of seryl-$tRNA_{UGA}$ greater than 60% of the total tRNA added is bound to ribosomes in response to UGA. Both species of seryl-tRNA are amino acylated with serine in the presence of an E. coli amino acyl-tRNA synthetase preparation. Two tRNA species (one of which is amino acylated with an E. coli synthetase preparation) which recognize UAG are being further investigated. Additional studies on aminoacylation of fractionated bovine liver tRNA with all 20 amino acids (assays may contain 18 unlabeled amino acids, one (^{3}H) and one (^{14}C) amino acids, or 19 unlabeled and one labeled amino acid) in presence of an E. coli amino acyl-tRNA synthetase preparation has demonstrated that some tRNA species (e.g. valine) are equally acylated with either E. coli or mammalian synthetases while others (e.g. glutamine or glycine) are acylated very poorly or not at all; and that apparently there is very little cross acylation.

Significance to Biomedical Research and the Program of the Institute:

A major unresolved question in biology is whether tRNA may play a role in cellular regulation and carcinogenesis. One approach to elucidating these problems is a study of the occurrence of suppressor tRNAs.

Proposed Course of Project:

To search for additional tRNAs which recognize nonsense codons and to set-up an E. coli protein synthesis system with mRNA containing nonsense mutations.

Publications

1. Hatfield, D., Portugal, F. H., and Caicuts, M.: Transfer RNA specificity in mammalian tissues and codon responses of seryl transfer RNA. Cancer Res. 31: 697-700, 1971.

Serial No. NCI-4765

1. Chemistry Branch, OASDC, Etiology
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on the Intracellular Replicative Genome of RNA Containing Tumor Viruses

Previous Serial Number: Same

Principal Investigator: John P. Bader

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	0.4
Other:	0.8

Project Description

Objectives:

To delineate the biochemical events which establish the replicative form of RNA-containing tumor viruses, and to identify that replicative form.

Methods Employed:

Cell culture, radioactive tracer techniques, density equilibrium and sedimentation rate centrifugations, isolation and purification of nucleic acids, molecular hybridization techniques, enzymological analyses, polyacrylamide gel electrophoresis.

Major Findings:

Earlier studies in this laboratory demonstrated a requirement for new DNA synthesis which along with other data indicated that a DNA complementary to viral RNA is synthesized and this DNA is the intracellular replicative genome from which progeny viral RNA's are synthesized. Recent experiments utilizing metabolic

analogues showed that the synthesis of viral DNA begins within one hour after attachment of virus to cells, and that reduplication of viral DNA occurs during the next 7 hours. Other experiments using a compound which disrupts the mitotic process (vinblastine sulfate) demonstrated that cellular divisions were not a requirement for virus reproduction, disproving a widely-held view that virus cannot reproduce without an intervening cellular division. Transformation of cells by Rous Sarcoma virus (RSV) also may occur without cellular division; morphological changes characteristic of Rous transformed cells are found after infection even though cellular divisions are prevented. Also, increased hexose uptake and increased hyaluronic acid synthesis, biochemical changes often found in malignant cells, are found in infected cells which have never divided after exposure to virus.

Significance to Biomedical Research and the Program of the Institute:

A more complete description of the reproductive process of RNA tumor viruses is essential to an understanding of tumorigenesis by these viruses, and may be useful in devising chemotherapeutic agents for the treatment of virus-caused neoplasms. The observation that cellular division is not an absolute requirement for transformation to malignancy resolves an important issue in tumorigenesis and may help to resolve the specific biochemical events which lead to malignancy.

Proposed Course of Project:

To extract from infected cells and identfy the DNA replicative forms of RNA-containing tumor viruses. The interaction of the viral DNA with cellular genes which leads to malignancy requires a biophysical demonstration of the intracellular viral DNA.

Publications

1. Bader, J. P., Metabolic requirements for infection by Rous Sarcoma virus III. The synthesis of viral DNA. Virology, (in press)

2. Bader, J. P., Metabolic requirements for infection by Rous Sarcoma virus IV. Virus reproduction and cellular transformation without cellular division. Virology, (in press)

Serial No. NCI-4717

1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Effect of Various Carcinogens, Drugs and Agents in the Environment on Genetic Transcription

Previous Serial Number: Same

Principal Investigators: Raphael Salomon, Ph.D. and Harry V. Gelboin, Ph.D.

Other Investigators: Andrew C. Peacock, Ph.D.

Cooperating Units: Protein Section, Chemistry Branch

Man Years:

Total:	0.8
Professional:	0.8
Other:	0.0

Project Description

Objectives:

To determine the effect of polycyclic hydrocarbons on protein synthesis of the microsomal membranes during aryl hydrocarbon hydroxylase (AHH) induction.

Methods Employed:

Separation of microsomal fraction from rat liver cells and from hamster embryo cells in culture. Solubilization of microsomal proteins by detergent and phospholipase or by phenol urea acetic acid, separation of the membrane proteins by disc gel electrophoresis.

Major Findings:

Microsomal proteins vary quantitatively in different preparations. To minimize the variability of microsomal preparations from BA treated cells and control cells of hamster embryos, a double-labeling technique was used. We found the use of ^{3}H-methyl-L-methionine and ^{35}S-methionine a very efficient

method to label proteins and found a significant increase in amino acid incorporation into BA treated microsomes. By the use of ^{3}H and ^{14}C-labeled amino acids we determined this increase to be 10-20% above control levels of incorporation. Separation by gel electrophoresis of phenol-urea-acetic acid soluble microsomal proteins revealed 13 major bands which were divided into 3 groups (bands 1-3, 4-9, 10-13). The second group contained about 70% of the radioactivity incorporated and was labeled much faster than the third group. The ratio of incorporation drops from 11 times after 1 hour to 3 times after 4 hours. However, the increase in amino acid incorporation was not defined to a limited number of proteins but was observed as an overall increase in protein synthesis during AHH induction. Labeling rat liver microsomes in vivo with ^{3}H-δ-amino levulinic acid which is the heme precursor of the P-450 showed a similar incorporation pattern in microsomal protein as was observed with labeled amino acids.

Significance to Biomedical Research and the Program of the Institute:

This study shows that benz(a)anthracene induction of AHH causes an increase of amino acid incorporation into the microsomal proteins of induced cells and thus points to the possibility of new enzyme synthesis in multiple P-450 proteins as a result of exposure to hydrocarbons. This relates to the role of these enzymes in carcinogenesis.

Proposed Course of Project:

To selectively inhibit protein synthesis in microsomes during AHH induction with inhibitors. This approach will show if the increase of protein synthesis is a requirement for AHH induction or the possibility of induction due to allosteric changes in the protein.

Publication

Younger, L.R., Salomon, R., Wilson, R.W., Peacock, A.C., and Gelboin, H.V.: Effects of polycyclic hydrocarbons on RNA synthesis in rat liver nuclei and hamster embryo cells. Mol. Pharmacol. (in press).

Serial No. NCI-4760

1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Role of Enzymatic Hydroxylation in Polycyclic Hydrocarbon Transformation and the Relation of Cell Transformation and Reversion to Enzyme Presence

Previous Serial Number: Same

Principal Investigator: Harry V. Gelboin, Ph.D.

Other Investigator: Leo Sachs, Ph.D.

Cooperating Units: Section of Genetics, Weizmann Institute of Science, Rehovot, Israel

Man Years:

Total:	0.4
Professional:	0.2
Other:	0.2

Project Description

Objectives:

To study aryl hydrocarbon hydroxylase in various cell lines under different physiological conditions and the relationship between the levels of enzymes and cell transformation and reversion.

Methods Employed:

Cell culture techniques, spectrophotofluorometry, recording spectrophotometry, fluorescent microscopy, and radioisotope studies are used in this project.

Major Findings:

A variety of inhibitors of enzyme activity have been tested for their effect on polycyclic hydrocarbon metabolism and transformation. 7,8-benzoflavone was found to prevent both. A chrysene and dibenzcarbazole derivative have also been found to be potent inhibitors of the enzyme system.

Significance to Biomedical Research and the Program of the Institute:

Polycyclic hydrocarbons represent major contaminants in the environment and are serious threats to the human organism in a highly industrialized society. The enzyme system dealing with these agents is aryl hydrocarbon hydroxylase, and our findings indicate that the enzyme system is responsible for the activation of the hydrocarbon to a toxic form. The enzyme system is also responsible for detoxification. The relationship and control of these two activities is important for understanding polycyclic hydrocarbon carcinogenesis.

Proposed Course of Project:

Variants from cells transformed by chemical carcinogens with a reversion of properties characteristic of transformation will be produced, and the properties of these variants will be analyzed. The carbazole and chrysene will be tested on these variants.

Serial No. NCI-4766

1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Cell Regulatory Controls of Microsomal Hydroxylase Metabolizing Polycyclic Hydrocarbons

Previous Serial Number: Same

Principal Investigators: Friedrich J. Wiebel, M.D. and Harry V. Gelboin, Ph.D.

Other Investigators: Hayden G. Coon, Ph.D. and Edwin J. Matthews

Cooperating Units: Laboratory of Cell Biology, GL&C, NCI

Man Years:

Total:	1.8
Professional:	1.3
Other:	0.5

Project Description

Objectives:

To study the control mechanisms regulating the synthesis and degradation of the microsomal enzyme system that metabolizes polycyclic hydrocarbons in a controlled environment such as tissue culture.

Methods Employed:

Cell culture techniques, fluorospectrophotometry, recording spectrophotometry, radioisotope techniques, and acrylamide gel electrophoresis.

Major Findings:

(1) The induction of aryl hydrocarbon hydroxylase (AHH) is continuously dependent on protein synthesis and requires RNA synthesis for an initial 2-hour period. Selective inhibition of ribosomal RNA synthesis by Actinomycin D before and during the presence of inducer does not interfere with the increase in enzyme activity. Higher levels of

Actinomycin D ranging from 0.06 to 0.12 µg/ml inhibit both the increase in enzyme activity and the appearance of heterogeneous RNA in the cytoplasm. Our results have shown that the early stages of enzyme induction require the synthesis of an RNA in the heterogeneous fraction whose formation, transport and expression is independent of concomitant ribosomal RNA synthesis and transport.

(2) Hybridization of somatic cells was used to study the control mechanism of basal level and inducibility of AHH activity. Intraspecific hybrids were formed between cells which differ in basal as well as inducible AHH. The results show that, depending on the type of cells fused, basal and induced AHH can be either suppressed below the levels of the less active parent or can be enhanced above the level of the more active parent. No examples were found in hybrid cells of a dissociation of basal and induced enzyme activity as reported for other inducible enzymes.

Significance to Biomedical Research and the Program of the Institute:

The enzyme system studied is involved in the metabolism of a large number of drugs, carcinogens, and environmental pollutants as well as a variety of endogenous compounds. It is a key factor in the response of the human organism to potentially toxic and carcinogenic foreign compounds.

Proposed Course of Project:

To study the mechanisms that regulate the level of enzyme activity. To devise means to control the activity of the enzyme system and the direction of alternative metabolic pathways. To determine the role of the enzyme complex in chemical carcinogenesis *in vitro*.

Honors and Awards

Invited Speaker, paper given in absentia, "On the relationship between transcription, translation, and ribosomal RNA synthesis during microsomal hydroxylase induction." XI Latin American Symposium on Protein Synthesis and Nucleic Acids, La Plata, Argentina, November 1971.

Publications

Conney, A.H. and Gelboin, H.V.: Antagonism and potentiation of drug action and the mechanism of microsomal enzyme induction. In Meyler, L. and Peck, H.M.: The Handbook of Drug-Induced Diseases. Amsterdam, The Netherlands, Excerpta Medica Foundation, Chapter 10, 1970 (in press).

Gelboin, H.V.: Mechanisms of induction of drug metabolism enzymes. In Brodie, B.B. and Gillette, J. (Eds.): Concepts in Biochemical Pharmacology: Handbook of Experimental Pharmacology. New York, Springer-Verlag, 1971, pp. 431-451.

Gelboin, H.V.: Mechanisms of induction of drug metabolism enzymes. In LaDu, Jr., B.N. (Ed.): Part 2, Drug Biotransformation; Environmental and Genetic Factors which Modify Drug Metabolism. Fundamentals of Drug Metabolism and Disposition. Baltimore, Md., Williams & Wilkins, 1971, pp. 279-307.

Wiebel, F.J., Matthews, E.J., and Gelboin, H.V.: On the realtionship between transcription, translation, and ribosomal RNA synthesis during microsomal hydroxylase induction. Symposium of the XI Latin American Symposium on Protein Synthesis and Nucleic Acids, La Plata, Argentina (in press).

Wiebel, F.J., Matthews, E.J., and Gelboin, H.V.: RNA synthesis dependent induction of aryl hydrocarbon hydroxylase in the absence of ribosomal RNA synthesis and transfer. J. Biol. Chem. (in press).

Serial No. NCI-4770

1. Chemistry Branch, OASDC Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: To Characterize and Quantitate the Metabolites of Benzo(a)pyrene formed In Vivo and In Vitro in Different Tissues and Species and to Search for an Active Carcinogenic Form

Previous Serial Number: Same

Principal Investigator: Nadao Kinoshita, Ph.D.

Other Investigators: Harry V. Gelboin, Ph.D., David Williams and Friedrich J. Wiebel, M.D.

Cooperating Units: None

Man Years:

Total:	1.8
Professional:	1.0
Other:	0.8

Project Description

Objectives:

To examine the metabolic profile of benzo(a)pyrene as it is metabolized in vitro by aryl hydrocarbon hydroxylase, a microsomal enzyme system.

Methods Employed:

UV and fluorescence spectrophotometry, thin layer chromatography, radioactive tracer techniques, enzymology.

Major Findings:

Besides the major metabolite, 3-OH-benzo(a)pyrene, a number of minor metabolites have been detected and identified and their separation on silica gel thin layer chromatography plates standardized. 3-Methylcholanthrene greatly induces the enzyme system in rat liver microsomes with an accompanying increase in the production of the 9,10-dihydrodiol. The

addition of 7,8-benzoflavone to the in vitro assay greatly reduces the relative production of this one metabolite as well as showing an overall inhibitory effect on the other metabolites.

Significance to Biomedical Research and the Program of the Institute:

It is important to determine which metabolite is responsible for the carcinogenic effect of the polycyclic hydrocarbons. The isolation of the active form of the carcinogen may be the initial step in finding the means of blocking its formation or rendering it harmless to the organism. An understanding of the metabolism of known carcinogens will eventually lead to the mechanism of carcinogenesis.

Proposed Course of Project:

To expand this study to other animals, other tissues and to determine the activity of the metabolites of benzo(a)pyrene and their response to the inhibitor, 7,8-benzoflavone.

Publication:

Williams, D., Leutz, J., Wiebel, F.J., and Gelboin, H.V.: The effect of polycyclic hydrocarbons in vitro on aryl hydrocarbon (benzo[a]pyrene) hydroxylase. Biochem. Pharmacol. 20: 2130-2133, 1971.

Serial No. NCI-4779

1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Evaluation of Various Compounds for their Effects as Inducers and Inhibitors of Aryl Hydrocarbon Hydroxylase Activity and Tumorigenesis

Previous Serial Number: Same

Principal Investigator: Harry V. Gelboin, Ph.D.

Other Investigators: Friedrich J. Wiebel, M.D., Nadao Kinoshita, Ph.D., N. P. Buu-Hoi, M.D., Janet Leutz

Cooperating Units: Institut du Radium, Paris

Man Years:

Total:	2.1
Professional:	1.8
Other:	0.3

Project Description

Objectives:

To develop methods to determine the activity of various compounds as inducers or inhibitors of carcinogen hydroxylating enzymes and polycyclic hydrocarbon tumorigenesis.

Methods Employed:

Cell culture techniques, spectrophotofluorometry, recording spectrophotometry, fluorescent microscopy and radioisotope studies and mouse skin tumorigenesis.

Major Findings:

Over fifty compounds have been tested for enzyme inducing activity in cell culture, and enzyme inhibitory activity in isolated microsomes and in cell culture. Some of these compounds have been examined for their effect on mouse skin tumorigenesis. Four of the compounds were found to have a

powerful inducing effect and several inhibited the enzyme. Three of these were found to significantly inhibit tumorigenesis.

Significance to Biomedical Research and the Program of the Institute:

Compounds that inhibit or induce the hydroxylating enzyme systems may be possibly used to alter the course of tumorigenesis.

Proposed Course of Project:

To reveal a variety of compounds that can regulate enzyme activity and polycyclic hydrocarbon tumorigenesis.

Publications

Gelboin, H.V.: Aryl hydrocarbon hydroxylase -- an enzyme system metabolizing polycyclic hydrocarbons. Report to the Panel on Polycyclic Organic Matter, National Research Council, Washington, D.C. (in press).

Gelboin, H.V.: Carcinogenesis, the environment, and gene action: New Horizons for Radiologists Lecture. Radiology 99: 251-263, 1971.

Gelboin, H.V.: Chemical carcinogens and the theories of their action. In Enciclopedia Medica Italiana, Rome, Italy (Ed. 2) (in press).

Gelboin, H.V.: Microsomal hydroxylases: Studies on the mechanism of induction and their role in polycyclic hydrocarbon action. Symposium Monograph, Environment and Cancer, M.D. Anderson Hospital, March 1971 (in press).

Gelboin, H.V.: Studies on the mechanism of microsomal hydroxylase induction and its role in carcinogen action. Revue Canadienne de Biologie (in press).

Gelboin, H.V. and Wiebel, F.J.: Studies on the mechanism of aryl hydrocarbon hydroxylase induction and its role in cytotoxicity and tumorigenicity. Ann. N. Y. Acad. Sci. 179: 529-547, 1971.

Wiebel, F.J., Leutz, J.C., Diamond, L., and Gelboin, H.V.: Aryl hydrocarbon (benzo[a]pyrene) hydroxylase in microsomes from rat tissues: Differential inhibition and stimulation by benzoflavones and organic solvents. Arch. Biochem. Biophys. 144: 78-86, 1971.

Serial No. NCI- 4521
1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Effect of Reducing Protein Synthesis on Carcinogen and Drug Metabolizing Systems

Previous Serial Number: None

Principal Investigators: Raphael Salomon, Ph.D. and Harry V. Gelboin, Ph.D.

Other Investigators: Andrew C. Peacock, Ph.D.

Cooperating Units: Protein Section, Chemistry Branch

Man Years:

Total:	1.1
Professional:	1.1
Other:	0.0

Project Description

Objectives:

To determine if polycyclic hydrocarbons can induce AHH when protein synthesis is suppressed and to determine whether the effect of inhibitors is localized in certain cell fractions.

Methods Employed:

Hamster embryo secondary cultures were treated with BA and cycloheximide. Protein synthesis was measured by the amount of labeled amino acid incorporated. Microsomes were separated from homogenates of cultured cells by sedimentation. Microsomal proteins were solubilized by heating in 1% SDS 0.1 M 2-mercaptoethanol and separated by gel electrophoresis.

Major Findings:

Cycloheximide in concentrations of 1 µg/ml culture medium reduced labeled amino acids incorporation by 85%. An increase in concentration of cycloheximide did not inhibit protein synthesis further, however, the increase in cycloheximide

concentration resulted in incorporation of amino acid into smaller proteins as was determined by gel electrophoresis. Under these conditions AHH was not induced in whole cell homogenate but the sediment of 105,000 Xg showed an increase of AHH activity even when 4 µg/ml cycloheximide were present in the culture medium. Microsomes from cells treated with cycloheximide showed double the AHH activity than microsomes from untreated cultures. The 105,000 Xg pellet accounts for only a small fraction of the endoplasmic reticulum and thus represents a small fraction of AHH present in the cells. In cultures treated with cycloheximide the increase of AHH in the microsomal fraction was not a result of a higher yield of this fraction and thus the remaining activity of the sediment below 8,000 Xg was not affected.

Significance to Biomedical Research and the Program of the Institute:

This study indicated that there is a small portion of the endoplasmic reticulum which responds in a different way to the administration of cycloheximide. In this membranous fraction the AHH induction may be due to an allosteric effect which is independent of protein synthesis. This might point to a system in humans which may be regulated by a similar mechanism.

Proposed Course of Project:

To study the mechanism which increases AHH activity in the absence of protein synthesis in the microsomal fraction and to increase the fraction of endoplasmic reticulum recovered in this fraction.

Serial No. NCI-4522

1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Molecular Mechanisms of Aryl Hydrocarbon Hydroxylase (AHH) Induction in Mammalian Cell Culture

Previous Serial Number: None

Principal Investigators: James P. Whitlock, M.D. and Harry V. Gelboin, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.8
Other:	0.0

Project Description

Objectives:

Characterization of AHH activity in a cloned cell culture derived from rat liver. Testing of polycyclic hydrocarbons and other biologically active compounds for AHH induction in this system. Use of inhibitors and reconstitution experiments to investigate the biochemical events immediately preceding the increase in AHH activity.

Methods Employed:

Cell culture; spectrophotofluorometry; recording spectrophotometry.

Major Findings:

A cloned cell line has been found in which AHH activity is highly inducible by polycyclic hydrocarbons, while basal activity is low. Kinetics of induction and effect of inhibitors on enzyme activity differ somewhat from other tissue culture systems. AHH activity is slightly inducible by phenobarbital.

Significance to Biomedical Research and the Program of the Institute:

This system represents an improvement over previous *in vitro* methods for studying AHH in that the cell population is homogeneous and retains certain of its *in vivo* characteristics. It potentially may be useful not only for studying the metabolism of environmental chemical carcinogens but also for investigating the detoxification of drugs and other biologically active compounds.

Proposed Course of Project:

Further investigation of the biochemical events relevant to induction of AHH activity.

Serial No. NCI-4523

1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Measurement of Aryl Hydrocarbon Hydroxylase (AHH) in Human Tissue

Previous Serial Number: None

Prinvipal Investigator: James P. Whitlock, M.D.

Other Investigators: Harry V. Gelboin, Ph.D. and Herbert L. Cooper, M.D.

Cooperating Units: National Institute of Dental Research Laboratory of Biochemistry

Man Years:

Total:	0.5
Professional:	0.3
Other:	0.2

Project Description

Objectives:

Examination of human tissue for presence of AHH activity.

Methods Employed:

Cell culture; spectrophotofluorometry.

Major Findings:

Studies just begun and are still in progress.

Significance to Biomedical Research and the Program of the Institute:

AHH has been implicated in the activation of potential chemical carcinogens and in their degradation to inactive metabolites. Enzyme activity has been correlated with tumorigenesis in experimental animals. AHH may be important in the process of chemical carcinogenesis in man; an easily-available source of human tissue with AHH activity could contribute significantly to the study of chemical

carcinogenesis and drug metabolism in humans.

Proposed Course of Project:

Continued search for AHH activity in human tissue. If a suitable source is found, studies involving the variation of AHH activity with respect to age, sex, race and previous exposure to drugs are envisioned. Drug metabolism in human tissue may also be more easily studied.

Serial No. NCI-4524

1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Effect of 7,8-Benzoflavone on Mouse Skin Tumorigenesis by 7,12-Dimethylbenz(a)-anthracene and Benzo(a)pyrene

Previous Serial Number: NCI-4780

Principal Investigators: Nadao Kinoshita, Ph.D. and Harry V. Gelboin, Ph.D.

Other Investigators: Barbara Shears

Cooperating Units: None

Man Years:

Total:	2.8
Professional:	0.8
Other:	2.0

Project Description

Objectives:

To examine the role of aryl hydrocarbon hydroxylase in the *in vivo* binding of 7,12-dimethylbenz(a)anthracene to biomacromolecules with the powerful inhibitor of the enzyme, 7,8-benzoflavone, and investigate the correlation between 7,12-dimethylbenz(a)anthracene carcinogenesis and its metabolism.

Methods Employed:

Animal carcinogenesis experiments, spectrophotometry, radioactive tracer techniques, enzymology.

Major Findings:

Aryl hydrocarbon hydroxylase is present and is inducible in mouse skin. 7,8-Benzoflavone, an inhibitor of the enzyme, markedly inhibits tumorigenesis by 7,12-dimethylbenz(a)-anthracene, but has either no effect or stimulates

benzo(a)pyrene tumorigenesis. Thus, the role of aryl hydrocarbon hydroxylase appears highly specific for each polycyclic hydrocarbon in respect to detoxification and/or activation of the hydrocarbon to a carcinogenic form. In parallel studies, we found that 7,8-benzoflavone significantly reduces the amount of 7,12-dimethylbenz(a)anthracene binding to mouse skin DNA, RNA, and protein, and the binding of benzo(a)pyrene to RNA and protein of mouse skin. 7,8-Benzoflavone exhibited a markedly lesser effect on the binding of benzo(a)pyrene to DNA. We have also shown by means of animal carcinogenesis experiments that 7,12-dimethylbenz(a)anthracene requires metabolic activation and that this activation is completed within 12 hours after the animal is exposed to the carcinogen. The level of DMBA tumorigenesis varies markedly in different mouse strains indicating either genetic differences in the profile of DMBA metabolite formation or that metabolic activation is necessary but not sufficient for DMBA tumorigenesis.

Significance to Biomedical Research and the Program of the Institute:

The active form of the carcinogen must be determined and its mode of action in the animal studied. An understanding of this may offer potential for the prevention or alteration of normal cell to malignant cell conversion.

Proposed Course of Project:

To study the metabolic profile of DMBA and benzo(a)pyrene to study which binding of carcinogen to biomacromolecules is related to normal cell to malignant cell conversion.

Honors and Awards

Invited Lecture, "Microsomal aryl hydrocarbon hydroxylases: Their induction and role in toxicity." 55th Annual Meeting, Pharmacogenetics Symposium, American Society for Pharmacology and Experimental Therapeutics, Federation of American Societies for Experimental Biology, Chicago, Illinois, April 1971.

Invited Lecture and Session Chairman, "Microsomal aryl hydrocarbon hydroxylases: Inhibition by 7,8-benzoflavone and their role in hydrocarbon toxicity and carcinogenicity." International Symposium on Drug Metabolism, Skokloster, Sweden, June 1971.

Invited Lecture, "Microsomal hydroxylases: Studies on the mechanism of induction and their role in polycyclic

hydrocarbon action." Symposium on Biological Hydroxylation Reactions of the Biochemical Society of the United Kingdom, Edinburgh, Scotland, July 1971.

Publications

Gelboin, H.V.: Microsomal aryl hydrocarbon hydroxylases: Their induction and role in toxicity. Proceedings of the American Society of Pharmacology and Experimental Therapeutics at the 55th Annual Meeting, Federation of American Societies for Experimental Biology, Chicago, Illinois, April 1971 (in press).

Gelboin, H.V.: Microsomal hydroxylases: Studies on the mechanism of induction and their role in polycyclic hydrocarbon action. Symposium on Biological Hydroxylation Reactions of the Biochemical Society of the United Kingdom, Edinburgh, Scotland, July 1971 (in press).

Kinoshita, N. and Gelboin, H.V.: The role of aryl hydrocarbon hydroxylase in 7,12-dimethylbenz(a)anthracene (DMBA) skin tumorigenesis: On the mechanism of 7,8-benzoflavone inhibition of tumorigenesis. Cancer Res. (in press).

Kinoshita, N. and Gelboin, H.V.: Aryl hydrocarbon hydroxylase and polycyclic hydrocarbon tumorigenesis: Effect of the enzyme inhibitor 7,8-benzoflavone on tumorigenesis and macromolecule binding. Proc. Natl. Acad. Sci. USA (in press).

Serial No. NCI-4783
1. Chemistry Branch, OASDC, Etiology
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Development of a Convenient Program for the Rapid Calculation of Sedimentation Coefficients in Linear Salt or Sucrose Gradients

Previous Serial Number: None

Principal Investigator: C. Wesley Dingman, M.D.

Other Investigator: M. Patricia Fisher

Cooperating Units: None

Man Years:
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description

Objectives:

To develop a convenient, easy to use, method for calculating standard sedimentation coefficients, $S_{20,w}$ of subcellular particles and macromolecules using zone sedimentation in preparative centrifuge rotors.

Methods Employed:

Starting with conventional mathematical approaches to the determination of $S_{20,w}$ from sedimentation data obtained using linear salt or sucrose gradients, empirical formulas for correcting the data for solvent viscosity changes as a function of temperature and sucrose concentration were devised. The validity of these formulas were then checked against standard published tables of sucrose viscosity as a function of temperature and concentration. In order to accommodate data derived from measurements in alkaline sucrose gradients, it was necessary first to determine the viscosity of such solutions ourselves before empirical formulas could be developed. Finally all the formulas were included in a program of sequential mathematical steps that could be easily translated for use on high speed digital computers.

Major Findings:

The program developed enables one to calculate sedimentation coefficients under standard conditions, $S_{20,w}$ from data derived by zone sedimentation in preparative centrifuge rotors, using any linear salt or sucrose gradient (up to 1 M in sucrose), and at temperatures ranging from $0^{o}C$ to $20^{o}C$. The accuracy obtained (when checked using molecules of known $S_{20,w}$) is within about 5% of the reported values obtained in analytical ultracentrifuges.

Significance to Biomedical Research and the Program of the Institute:

The mathematical procedures developed should aid investigators in a wide variety of biomedical fields to determine easily the sedimentation characteristics of the particles or molecules they may be studying.

Proposed Course of Project:

To publish these findings.

Publications

Dingman, C. W.: A convenient program for the rapid calculation of sedimentation coefficients in linear salt or sucrose gradients. Anal. Biochem. (in press).

Serial No. NCI-4784
1. Chemistry Branch, OASDC, Etiology
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Role of Molecular Conformation in Determining the Electrophoretic Properties of Polynucleotides in Agarose-Acrylamide Gels

Previous Serial Number: Same

Principal Investigator: C. Wesley Dingman, M.D.

Other Investigators: M. Patricia Fisher, Tsuyoshi Kakefuda, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	1.3
Professional:	0.6
Other:	0.7

Project Description

Objectives:

To determine whether polyacrylamide gel electrophoresis under controlled conditions would be a useful technique for defining the structure of newly isolated, synthesized, or discovered polynucleotides.

Methods Employed:

A number of different polynucleotides having known structures were isolated from viral, bacterial, and mammalian sources. Low molecular weight DNA fragments were isolated from E. coli by sonicating and centrifuging purified high molecular weight DNA. The molecular weight of these linear fragments was determined by both electron microscopy and zone sedimentation in sucrose gradients. These various polynucleotides were then examined for their electrophoretic behavior in composite agarose-acrylamide gels under different conditions of temperature, gel concentration, and voltage gradient.

Major Findings:

We have further explored the relationship between the retardation coefficient, K_R (where log mobility = log mobility at zero gel concentration minus K_R multiplied by the gel concentration) and molecular weight. In general, for single-stranded polynucleotides, we found that Mol. Wt. = $A.K_R^{1.5} + B$, a result that might have been expected on the basis of theoretical arguments regarding acrylamide gel electrophoresis developed by others. Double stranded, linear, polynucleotides above about 3×10^5 daltons, on the other hand, migrate in gels as if they all had molecular radii of about 35 A. This value agrees well with low angle, X-ray scattering measurements of the cross-sectional radii of these molecules in aqueous solutions made by others and further confirms our belief that these molecules migrate "end on" in acrylamide gels. This finding would explain why the mobilities of high molecular weight, double-stranded, linear, polynucleotides are unrelated to their molecular weights. The data for linear, single-stranded, polynucleotides, and low molecular weight, linear double-stranded, polynucleotides indicated that the relationship between log mol. wt. and mobility was not linear as previously thought, but showed upward and downward curvature at low and high mobilities, respectively, as would be predicted from the above mentioned relationship between K_R and Mol. Wt.

Significance to Biomedical Research and the Program of the Institute:

The results of this project will enable many investigators in both virology and nucleic acid metabolism to ascertain, rather inexpensively, the major structural aspects of newly isolated polynucleotides of biomedical significance. Furthermore, the techniques developed under this project are particularly useful when the amount of material available for investigation is very small. Thus, it is hoped that these procedures will aid in defining the nature of the genome of newly isolated viruses of oncogenic significance as well as prove useful in defining the various products of "reverse transcriptase" activity in both *in vivo* and *in vitro* studies.

Proposed Course of Project:

To publish these results.

Publications

Fisher, M. P. and Dingman, C. W.: The role of molecular conformation in determining the electrophoretic properties of

polynucleotides in agarose-acrylamide gels. Biochemistry 10: 1895-1899, 1971.

Dingman, C. W., Fisher, M. P., and Kakefuda, T.: The role of molecular conformation in determining the electrophoretic properties of polynucleotides in agarose-acrylamide gels, II. Biochemistry (in press).

Dingman, C. W.: The relationship between the molecular weight of polyribonucleotides and their electrophoretic mobility in agarose-acrylamide gels. In Broda, E., Locker, A., Springer-Lederer, H. (Eds.): Proc. First European Biophysics Congress, Wien, Austria, Vienna Medizinischen Akademie, 1971, Vol. 1, pp. 251-258.

Serial No. NCI-4785

1. Chemistry Branch
   OASDC, Etiology
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Role of DNA Repair Mechanisms In The Etiology of Cancer

Previous Serial Number: NCI-4785

Principal Investigators: Rufus Day, III, Ph.D., C. Wesley Dingman, M.D.

Other Investigators: M. Patricia Fisher, Minoru Ishizawa, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.4
Professional:	1.2
Other:	0.2

Project Description

Objectives:

To learn more about DNA repair mechanisms in mammalian cells and their role in carcinogenesis. In particular, to determine whether agents known to be carcinogens also provoke or interfere with cellular repair systems.

Methods Employed:

Ultraviolet light (UV) of 253.7 nm specifically causes well defined, repairable, photochemical alterations in both the DNA and the RNA of cells at doses which cause little alteration in proteins, lipids, and carbohydrates. To study the repair of UV damaged DNA, it is important to use an experimental system in which possible complicating effects due to damaged RNA are reduced. These effects are actually excluded during the cellular repair of extracellularly UV-irradiated viruses containing only DNA and proteins. Therefore, we are currently studying the applicability of using such a virus, Human Adenovirus 2, as a probe to measure repair according to the following rationale. The DNA of a UV-irradiated virus is subject to the action of the repair system

in the host cell it infects. If the cellular repair system is damaged or interfered with, the repair of the damaged infecting virus is decreased. This decrease is manifested experimentally as a decrease in the ability of the virus to initiate and perpetrate a macroscopic area of cell lysis on a cell monolayer. Such a detection system should allow us relatively rapid, sensitive, and quantitative screening of a number of carcinogens for anti-repair activity, and of a number of cell lines for genetic differences in the efficiency of DNA repair mechanisms. We also plan to follow the kinetics of DNA repair by incubating Adenovirus infected cells with 5-Bromodeoxyuridine (5-BUdR) to allow the incorporation of 5-BUdR into repaired patches in the UV-irradiated Adenovirus DNA. It is known that DNA containing 5-BUdR is susceptible to breakage by irradiation at 313.2 nm whereas normal DNA is not. Such breaks are detected by measuring the decrease in size of the isolated DNA after irradiation at 313.2 nm. By watching the DNA size become smaller as repair time increases the time course of the repair process can be measured.

Major Findings:

We have made some progress in the development of a tissue culture system for measuring cellular competence to repair UV-damaged DNA using UV-damaged Adenovirus as the test DNA and examining for host-cell reactivation which will be interpreted as a measure of host-mediated DNA repair.

Significance to Biomedical Research and the Progress of the Institute:

An evaluation of the role (if any) of DNA repair mechanisms in conferring resistance or susceptibility to mutagenesis and carcinogenesis is an important facet of any overall program to understand some of the molecular mechanisms underlying carcinogenesis. Physical, viral, and chemical carcinogens all are known to affect the structural integrity of a cell's genetic apparatus. It is a long range goal of these projects to determine whether or not such effects are important to an understanding of carcinogenesis, but it is expected that an understanding of genetic repair mechanisms, in general, will benefit many areas of biomedical research.

Proposed Course of Project:

To pursue the goals outlined above in Objectives.

Serial No. NCI-4787
1. Chemistry Branch,
OASDC, Etiology
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mode of Replication of RNA Tumor Viruses

Previous Serial Number: NCI-4787

Principal Investigator: Tsuyoshi Kakefuda, M.D., Ph.D.

Other Investigators: C. W. Dingman, M.D., Tina M. Bak

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description

Objectives:

To observe the sequential events of gene replication of RNA tumor viruses in host cells.

Methods Employed:

Tissue culture cells are infected with RNA tumor viruses and pulse-labeled with ^{3}H-thymidine at different post-infection times. The cells are fixed and prepared for light and electron microscopic autoradiography. The control cells are not exposed to virus. Photopositive grains are counted in each group and compared. To determine the site and time sequence of reverse transcription of the viral RNA, subcellular components, such as cell membranes, microsomes, polyribosomes, and mitchondria are isolated from previously infected and labeled cells. For in vitro transcription studies, subcellular organelles are isolated from virus infected cells without pre-incubation with radioactive precursors. These fractions are then analyzed for template activity in the form of RNA-directed and DNA-directed DNA synthesis. Nucleic acids are then extracted and analyzed by Cs_2SO_4 equilibrium centrifugation, polyacrylamide gel electrophoresis, and hybridization with native viral RNA.

Major Findings:

The autoradiographs of virus infected cells showed a significantly higher number of grains in the cytoplasm than uninfected cells. The grains appeated at the surface membrane in the early stages of infection. The isolated surface membrane from virus infected cells showed a higher incorporation of ^{3}H-thymidine than uninfected cells. There were no differences in the radioactivity of the mitochondria. The surface membrane of infected cells showed a markedly higher activity for DNA synthesis *in vitro*. Cesium sulphate equilibrium centrifugation analysis of the extracted nucleic acids suggested that the *in vitro* DNA synthesis was associated with the production of a nybrid of DNA and viral RNA rather than double stranded DNA. The DNA products were the same when whole cytoplasm was used in place of isolated cell membranes. The mitochondria had no template activity in this system. These results indicate that the reverse transcription of the viral RNA is closely associated with the cell membrane only a few hours after infection. Formation of double stranded DNA presumably takes place elsewhere.

Significance to Biomedical Research and the Program of the Institute:

Incorporation of viral genome into the genetic system of the host cell appears to be one of the steps in viral carcinogenesis. The mechanism of the replication and the nature of intermediate products of the viral RNA in the cells is, however, largely unknown. The autoradiographic and biochemical approach used in the present experiments were found to be useful tools to elucidate the sequential events which occur in infected cells. The involvement of the surface membrane in the early stage of transcription is of particular interest since some alteration of its metabolism or structure by drugs or immunological treatment may offer some potential for the modification of malignant transformation by RNA tumor viruses.

Proposed Course of Project:

To extend the observations described above using different cell lines (including human cells), viruses, and other purified subcellular components. The effect of various drugs which may alter the metabolism of nucleic acids and those which may affect membrane structure will also be studied.

Publications

1. Hatanaka, M., Kakefuda, T., Gilden, R.V., and Callan, E.A.O.: Cytoplasmic DNA synthesis induced by RNA tumor viruses. Proc. Nat. Acad. Sci. 68: 1844-1847, 1971.

Serial No. MCI-4718
1. Chemistry Branch, DASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Disc Electrophoresis - A Study of the Fractionation of Protein Mixtures on Cylindrical Columns of Polyacrylamide Gels

Previous Serial Number: Same

Principal Investigator: Arthur T. Ness, Ph.D.

Other Investigators: Julia V. Pastewka and Andrew C. Peacock, Ph.D.

Cooperating Units: None

Man Years:

Total:	2.1
Professional:	2.1
Other:	0.0

Project Description

Objectives:

There are five objectives: (a) study of the variables involved in the fractionation of protein mixtures; (b) identification of the fractions and their biochemical significance; (c) determine and interpret the significance of the changes produced by systems known to alter the secondary and tertiary structures of proteins; (d) study of protein-protein interactions and related variables; (e) study of the effect of hormones and other factors on the induction and rate of biosynthesis of proteins by various cells and tissues.

Methods Employed:

1. The disc polyacrylamide gel electrophoresis technique as developed by Ornstein and Davis and as modified by others for particular systems.

2. The use of other fractionation techniques such as column chromatography, preparative gel electrophoresis, ultracentrifugation, etc., to obtain homogeneous, unaltered protein entities to aid in the identification of the components obtained

in disc gel electrophoresis and in the interpretation of the results obtained in studies outlined in the broad objectives.

3. The technique of isoelectric focusing in disc acrylamide gel systems. This establishes a pre-selected pH gradient range (wide or narrow) and permits the separation of proteins of isoelectric point difference of 0.02 pH unit.

Major Findings:

1. The study of the subunit structure of haptoglobins was continued. The haptoglobins are made up of an unknown assemblage and structure of subunit polypeptides as indicated for the three common main phenotypes: Hp 1-1 (α1 and β subunits), Hp 2-2 (α2 and β), Hp 2-1 (α1, α2, and β). The subunits are held together principally by disulfide bonds which can be split by reductive cleavage procedures. The separated α1, α2, and β subunits can be resolved by disc polyacrylamide gel electrophoresis. However, we have not yet resolved the problem of a suitable gel electrophoretic system which will permit reliable quantitation of the α1 and α2 subunits. The investigation is being continued.

2. A reliable disc polyacrylamide gel electrophoretic procedure for haptoglobin subtyping has been developed and a manuscript prepared for publication.

3. The dye referred to as "Stains-All" or SA (Eastman 2718) has been found to be very useful by showing differential color staining of RNA and DNA electrophoretically resolved on gels.

Application of this dye to proteins separated by gel electrophoresis indicated a differential color staining of normal proteins versus phosphoproteins. This property suggested an important use of SA in the study of milk and mammary tissue proteins.

The development of a satisfactory procedure has presented difficulties, due to (1) the light sensitivity of the dye, (2) the interference by sodium dodecyl sulfate (SDS) used in the electrophoretic systems, and (3) the availability of only a few types of phosphoproteins and these not in pure form (including caseins).

Some useful procedures have been developed, and the investigations are being continued.

Significance to Biomedical Research and the Program of the Institute:

The high resolution and sensitivity of the disc electrophoretic technique permit study of the occurrence and significance of genetic variants of protein entities and also the analysis of subcellular fractions to determine the site or sites of protein synthesis and a study of the biochemical factors in protein synthesis. The technique may also permit the detection and assessment of physiological changes associated with the development of malignancy.

Proposed Course of Project:

1. Continue the study of the isolated polymeric proteins of Hp 2-1 and Hp 2-2 to determine the subunit assemblages of these proteins.

2. Continue the study of the disc gel techniques to evaluate the qualitative and quantitative changes in mammary tissue proteins produced by hormonal and carcinogenic agents.

Serial No. NCI-4519
1. Chemistry Branch, OASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Electrophoretic Characteristics of DNA

Previous Serial Number: NCI-4781

Principal Investigator: Robert S. Zeiger, M.D., Ph.D.

Other Investigators: Andrew C. Peacock, Ph.D., Raphael Salomon, Ph.D., and C. Wesley Dingman, Ph.D.

Cooperating Unit: Molecular Carcinogenesis Section, Chemistry Branch

Man Years:

Total:	1.7
Professional:	1.7
Other:	0.0

Project Description

Objectives:

Study the electrophoretic characteristics of DNA using agarose-acrylamide composite gels and evaluate the binding of DMBA to mouse DNA.

Methods Employed:

DNA is analyzed by electrophoresis in composite agarose-acrylamide gels, by thermal denaturation, by zone sedimentation in sucrose gradients. Isotopic experiments include labeling of HeLa DNA with ^{14}C-thymidine, ^{3}H-deoxyguanosine and ^{3}H-cytidine and mouse skin *in vivo* with ^{3}H-dimethylbenz(a)anthracene (DMBA). Nucleosides obtained from the enzymic degradation of ^{3}HDMBA-labeled mouse DNA are separated by paper chromatography using various solvent systems. Radioactivity is measured by liquid scintillation counting.

Major Findings:

We have shown that deoxyribonucleic acid (DNA) extracted from mice either by the phenol or Marmur methods was separated electrophoretically into two bands on composite polyacrylamide-

agarose gels. On a 2.5% polyacrylamide-0.5% agarose slab gel a fast-moving (FMB) and minor slow-moving (SMB) band were observed in both newborn and adult mice but not in guinea pig, rat, or man. The SMB migrated 0.5 mm/hr slower than the FMB at 200V and comprised 9% of the total cellular DNA. Both bands were degraded by DNAase but not RNAase and incorporated methyl-^{3}H-thymidine proportionally to their concentrations.

We demonstrated conclusively that mouse satellite and main-band DNA as they are defined by CsCl ultracentrifugation are identical to the slow and fast moving bands, respectively. In addition, the buoyant densities of the slow and fast-moving bands are equivalent to satellite and main-band DNA densities, respectively. These results indicate that the SMB observed on gel electrophoresis of mouse DNA is composed of low density DNA and is equivalent to mouse dA-dT rich satellite DNA obtained by CsCl centrifugation.

We have been also able to separate the low density dA-dT rich satellite DNA of the crab from its higher density main-band DNA by gel electrophoresis and demonstrate by methods used above that the low density dA-dT rich crab satellite DNA migrates in a discrete band (about 12% of the total DNA) electrophoretically slower than the main-band DNA. This electrophoretic fractionation of mouse and crab satellite DNA appears not to be a function of molecular weight since crab satellite and main-band DNA were shown by zone sedimentation in sucrose gradients to have similar molecular weights.

The effects of nucleotide composition and molecular weight of DNA upon its electrophoretic mobility in composite polyacrylamide-agarose gels were studied. DNA from nine microorganisms with different G-C content (guanine-cytosine) varying from 25 to 67% was extracted by a modified Marmur procedure. DNA was designated "unsheared" (DNA derived from the conventional extraction procedure but with a deliberate attempt to minimize shear forces) and "sheared" (DNA obtained by passing a solution of "unsheared" DNA ten times through a 27 gauge needle). By plotting the electrophoretic mobilities of DNAs within either the unsheared or sheared groups against their respective G-C contents, a direct linear relationship between G-C content and electrophoretic mobility was observed. DNAs with higher G-C contents migrated electrophoretically greater in composite gels. Additionally, the curve describing the mobilities of sheared DNA though parallel to that of unsheared DNA was shifted to the right by ten percent, representing a faster mobility compared to unsheared DNA. Within either the unsheared or sheared DNA groups the molecular weights of the DNAs, as determined by zone sedimentation in sucrose gradients,

were very similar. On the other hand, the molecular weights of sheared DNA were approximately 70 to 80% lower than that of unsheared DNA and probably contributed to the 10% greater mobility of sheared DNA. Therefore, though DNA mobility in composite gels is related directly to base composition, it is affected also by molecular weight. The influence of base composition on DNA mobility is easily recognized by comparing DNAs with similar molecular weights. The effect of base composition upon the secondary structure of DNA in relation to the mobility of DNA in composite gels, was probably a factor in this result.

We have shown that gel electrophoresis is a very accurate method to evaluate the products obtained from the thermal denaturation of DNA. DNA from HeLa cells labeled *in vitro* for 24 hours with ^{14}C-thymidine and ^{3}H-deoxyguanosine was extracted by a Marmur technique and denatured thermally in 0.1 x SSC. At one degree temperature intervals (in the critical temperature range) aliquots were removed and quenched rapidly in ice. Portions of the aliquots were measured spectrophotometrically at A_{260} or subjected to polyacrylamide gel electrophoresis. As denaturation begins, as noted by hyperchromicity, a new very slowly migrating band (denaturation band) first becomes apparent. The denaturation band showed a T_m of 77.5°C ± 0.5°C which was identical to that found spectrophotometrically. The relative quantity of DNA within the denaturation band reflected very accurately the degree of DNA thermal denaturation. By labeling HeLa deoxyguanosine with ^{3}H and thymidine with ^{14}C we were able to demonstrate that the most rapidly denaturing DNA was relatively A-T rich and the slowest denaturing DNA was relatively G-C rich, reflecting the relative hydrogen bonding potential of the respective base pairs (three hydrogen bonds in a G-C pair versus two in an A-T pair). In addition, the entire native HeLa DNA band migrated electrophoretically along a continuum (gradient) from relative A-T rich to relative G-C rich DNA.

We have previously shown *in vivo* that ^{3}H-DMBA bound to mouse epidermal satellite DNA to the same extent as main-band DNA. Further studies have shown that ^{3}H-DMBA binds covalently to the nucleosides of mouse DNA. Preliminary studies suggest that the purine nucleosides are the major binding site for the DMBA.

Significance to Biomedical Research and the Program of the Institute:

The electrophoretic technique provides a rapid method for the separation of mouse satellite DNA and a reliable assay for ascertaining differences in metabolic activity of these two species of DNA in normal and malignant tissues. It additionally

may serve as a mode of separating and investigating specific sites of the genome.

Proposed Course of Project:

To investigate the structural properties of DNA by gel electrophoresis and study the mode of binding of chemical carcinogens to DNA.

Publications

Zeiger, R. S., Salomon, R., and Peacock, A. C.: Isolation of mouse satellite deoxyribonucleic acid by composite polyacrylamide gel electrophoresis. Biochemistry 10: 4219-4223, 1971.

Zeiger, R. S., Salomon, R., Kinoshita, N., and Peacock, A. C.: The binding of 9,10-dimethyl-1,2-benzanthracene to mouse epidermal satellite DNA in vivo. Cancer Research, (in press).

Serial No. NCI-4721
1. Chemistry Branch, OASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on Electrophoretic Techniques

Previous Serial Number: Same

Principal Investigator: Andrew C. Peacock, Ph.D.

Other Investigator: Roswell A. Reed

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	1.2
Other:	0.0

Project Description

Objectives:

To study electrophoretic properties of nucleotides. To develop analytical procedures for the estimation of nucleotides in samples suitable for RNA analysis and to estimate the base composition of RNA species isolated only by gel electrophoresis.

Methods Employed:

Standard electrophoretic techniques with the use of chromatographic and ultracentrifugal methods to obtain samples suitable for study. Radioisotope counting of ^{14}C and tritium. Optical scanning of stained and unstained gels. Tissue culture of malignant cells.

Major Findings:

Studies on base composition of rapidly labeled RNA. Continued experience with the methodology which we had used earlier showed that the method was generally unreliable, the primary difficulty being the low rate of incorporation of guanosine. The labeling protocols were changed so that each of four tubes contained ^{14}C-methionine and either C, A, G, or U with the tritium label.

By this means, much improved reproducibility has been obtained, but definite figures for the base composition analysis are not yet available.

Significance to Biomedical Research and the Program of the Institute:

The role of very heavy ribonucleic acids characteristically found in rapidly dividing tissues has been speculated to be either (a) messenger RNA or (b) regulatory RNA. Characterization of these RNA species in normal and malignant tissues may show light on differences in the control mechanisms in malignant cells.

Proposed Course of Project:

Techniques developed thus far through the use of HeLa cells will be extended and applied to measurements made in mammary tissue. Estimates will be made of possible differences in base composition influenced by hormonal composition of the medium. We will study the influence of the mammary tumor virus on the production of this RNA, and in particular, we will try to use the base composition to help identify the presence of mammary tumor virus in suitably infected tissues.

Serial No. NCI-4731
1. Chemistry Branch, OASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Electrophoretic Analysis of RNA

Previous Serial Number: Same

Principal Investigator: Andrew C. Peacock, Ph.D.

Other Investigator: Sylvia L. Bunting

Cooperating Units: None

Man Years:

Total:	1.3
Professional:	1.3
Other:	0.0

Project Description

Objectives:

To devise methods for extraction of RNA and DNA from malignant cells in a manner best fitting to preserve the native state and to provide samples suitable for electrophoretic analysis.

Methods Employed:

Cultures of HeLa cells, assay of radioactivity, rapid isolation of RNA, and electrophoretic analysis of RNA.

Major Findings:

We have continued our studies on the analysis of synthesis of RNA in the nucleus in HeLa cells.

1. Extraction procedures

The amount and kind of RNA which is extracted from the nucleus was found to depend on the salt concentration and the pH employed during extraction. The yield of intact RNA increased as the pH of extraction was increased from pH 5 to pH 8 approximately 2-fold. At each pH the heavier, more rapidly labeling RNAs were lost as the salt concentration increased from 0.01 M to 0.5 M. In addition, the use of chloroform in the

extraction procedure for whole cell RNA was found to be disadvantageous, particularly at higher pHs. The alterations in the amount and type of RNA obtained from the nucleus as it is affected by salt concentration may come about through the differing behavior of the detergent under these conditions.

2. Labeling of DNA from RNA precursors

The distribution of label from uridine and methionine into RNA and DNA varied with the purity of material used as precursors. Extensive DNA labeling, which we reported earlier, was not observed when very fresh stocks of uridine and methionine were used.

3. Labeling conditions

The use of methionine-free medium in which to study the methylation of ribosomal RNAs has previously been reported to be of value. We find, however, that when the medium became depleted of methionine, mature forms of ribosomal RNA were not produced, even though other forms of nuclear RNA were not significantly altered. The addition of ethionine to the medium resulted in the failure of the cells to produce ribosomal RNA as was observed during methionine deprivation.

Significance to Biomedical Research and the Program of the Institute:

The rapidly labeled RNAs of high molecular weight that occur in the nucleus of a cell occupy a central role in its physiology. These findings provide a new insight into the relationships that exist between these species. In particular, most of the species previously thought to be ribosomal may now be considered to play either a regulatory or an informational role. A clarification and resolution of the issues presented by this report will provide a much deeper insight into the metabolism of the cell.

Proposed Course of Project:

We hope to clarify whether the RNAs are informational or regulatory, and we wish to study the process by which the cell controls the transition from one form to another. Since the conversion of ribosomal RNA from its precursors is correlated with cell growth, we plan to determine whether the conversion is a cause or a result of increased cellular proliferation.

Serial No. NCI-4782
1. Chemistry Branch, OASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Hormonal Effects on Normal and Malignant Breast Tissue in Culture

Previous Serial Number: Same

Principal Investigator: Marie R. Green, Ph.D.

Other Investigators: Andrew C. Peacock, Ph.D. and Julia Pastewka

Cooperating Unit: None

Man Years:
Total: 1.2
Professional: 1.2
Other: 0.0

Project Description

Objectives:

To study the response of normal and malignant breast tissue in culture to hormones in terms of RNA and DNA synthesis and the production of proteins (particularly milk proteins), and to evaluate the role of prolactin in carcinogenesis.

Methods Employed:

Culture of mammary explants, isolation of RNA and DNA, electrophoretic analysis of RNA, isolation of milk proteins, assay of radioactivity, microscopic examination of explants, autoradiography.

Major Findings:

Explants of mammary tissue of mice in midpregnancy cultured on a synthetic medium with insulin and hydrocortisone respond to the addition of prolactin by increased synthesis of proteins. Previous investigations had shown that the amounts of material secreted into the alveolar lumen (estimated from the appearance of histological sections) varied with the concentration of

prolactin in the medium. We have studied the nature and amount of the proteins made by the explants in response to prolactin by electrophoretic analysis of the proteins. One band in particular increased in proportion to the amount of prolactin present in the medium during a five day culture. As little as 5 ng/ml ovine prolactin was required to produce a measurable increase in the accumulation of this band. In order to relate this protein to the proteins of milk, mouse milk has been fractionated and electrophoresed at pH 2.7 and pH 7.2. The proteins from post-mitochondrial supernatants of mouse mammary explants have been partially characterized in terms of mouse milk. The band which accumulates in response to prolactin is thought by preliminary analysis to be a form of casein. Measurement of this band may form the basis for a sensitive assay for prolactin.

Electrophoresis of proteins from post-mitochondrial supernatants of explants at pH 2.7 separates milk proteins from other tissue proteins into distinct groups. We are studying the effects of various inhibitors of RNA synthesis to determine in what respects the sensitivity of DNA coding for proteins in general, differs from that coding for the so-called differentiative or "luxury proteins."

During the course of the work on electrophoresis of milk proteins we have studied a stain which seems to have special staining properties to proteins containing phosphorus. We have investigated optimal conditions under which this stain may be used. Mouse milk has been electrophoresed on gels and the phosphorus containing caseins have been distinguished from whey proteins by means of this stain.

Significance to Biomedical Research and the Program of the Institute:

The mouse mammary explant system as a bioassay for prolactin offers advantages to other systems such as the pigeon crop assay previously used in terms of sensitivity, availability, and ease of animals kept and numbers of animals used. Assay of proteins by the method we describe offers more precise quantitation than examination of histologic sections. No radioactive isotopes are required. Samples may be frozen.

A stain particularly sensitive to phosphorus containing proteins when used in conjunction with stains ordinarily used for staining proteins on gels following electrophoresis would be of great utility in localizing and distinguishing phosphoproteins from non-phosphorus containing proteins. In addition to the application of such a technique to the study of milk proteins studies with cyclic AMP have shown that phosphorylation and

dephosphorylation of enzymes and other protein molecules is a key mechanism in the control of cellular metabolism. A technique applicable to the study of phosphorylated proteins in general would probably find wide application in studies of the control of cellular metabolism.

Proposed Course of Project:

Because mammary tissue in culture responds to prolactin in terms of increased RNA synthesis and milk protein production, this system may be used as a sensitive bioassay for measuring amounts of prolactin present in blood. Blood from persons under extended hormone therapy for contraception and of patients who have had breast surgery for malignancy whose treatment included further surgery such as ovariectomy, adrenalectomy or hypophysectomy would be assayed for prolactin levels (under contract). Biopsy material of breast tissue from malignant tumor-bearing hosts (animal or human) may be grown and examined for responsiveness to hormones for retrospective and prognostic purposes. The effect of individual hormones on such tissue, whether the malignancy is initiated by viruses or chemicals may be determined in culture.

Publications

Green, M. R., Bunting, S. L., and Peacock, A. C.: Changes in labeling pattern of RNA from mammary tissue as a result of hormone treatment. Biochemistry 10: 2366-2371, 1971.

SUMMARY REPORT

EXPERIMENTAL PATHOLOGY BRANCH
July 1, 1971 through June 30, 1972

During the past fiscal year the Experimental Pathology Branch began a major transition in its function and organization which will continue into the next fiscal year. This transition relates principally to the assumption of more clearly defined responsibilities for the development of major portions of the National Cancer Institute's program dealing with the prevention of cancer caused by chemical and physical means. The two primary responsibilities of the Branch are (1) the development of systems for the assay of environmental carcinogens and their utilization for detecting chemical carcinogens to which humans are exposed, and (2) the development of animal models of the main forms of human cancer and their utilization to study the etiology and pathogenesis of these forms of cancer. In general, the responsibility for the first function is performed by the Bioassay Contract Segment which is directed by the staff of the Carcinogen Screening Section; whereas, the second function is the responsibility of the Biological Models Contract Segment, by members of the Bioassay Section. Members of this Branch also serve on the Colon Cancer, Lung Cancer, and Information and Resources Segments of Carcinogenesis, on the Breast Cancer Virus Working Group in Viral Oncology, and in the Experimental Therapeutics Contract Review Committee in Chemotherapy. At the same time, they carry on research in their intramural laboratories.

At the present time, the intramural laboratories of the Experimental Pathology Branch are composed of the following subdivisions:

A. Office of the Chief

(1) Histopathology Unit: (a) Plans, organizes, and conducts investigations on the pathology of processes required for the development of the carcinogenic response to chemical carcinogens using optical and electron microscopic methods; (b) develops and monitors standards of pathology for carcinogenesis studies; (c) provides assistance and advice to staff of the Carcinogenesis Area on problems relating to histopathology and ultrastructure of tumors; and (d) provides histology service for the Carcinogenesis Area.

B. Bioassay Section: (1) Plans, organizes, and conducts research to develop and select new and improved methods and techniques for the bioassay of carcinogenic agents; (2) defines and standardizes bioassay systems to obtain quantitation of carcinogenic responses; (3) defines conditions of species and organ susceptibility, age, sex, and genetic factors affecting the specificity of carcinogenic agents and the ways in which these factors influence the interaction of carcinogens with target sites.

C. Carcinogen Screening Section: (1) Develops and monitors screening programs for the detection of carcinogenic hazards of environmental

chemical agents; (2) investigates the toxic, pharmacologic, and carcinogenic activity of selected chemicals or mixtures of environmental significance; and (3) explores metabolic pathways of selected test chemicals in different animal species.

The contract related segments for which the members of the Branch have primary responsibility are the following:

A. Bioassay Segment: (1) Tests chemicals to which humans are exposed to determine their carcinogenicity in experimental animals, (2) develops improved approaches to screening chemicals for carcinogenicity through better standardization of test conditions, more rapid screening procedures, etc. (3) determines the human relevance of the results of bioassays.

B. Biological Models Segment: (1) Develops animal models of major forms of human cancer such as pancreas, prostate, breast, bladder, kidney, and childhood cancers; (2) utilizes these models to study the etiology and pathogenesis of human cancer; (3) investigates stages in the pathogenesis of these forms of cancer which are amenable to preventive actions.

Future plans call for steps which will bring the intramural organization of the Experimental Pathology Branch more in line with the responsibilities for the collaborative program. For example, it is hoped to have scientists on the intramural staff working on each of the organ systems for which the Biological Models Segment has responsibility so that they are properly qualified to bear a major responsibility in the planning of collaborative programs on carcinogenesis in these organs. Similarly, the Bioassay Segment which has a responsibility for testing programs on a diverse variety of materials such as natural products, industrial products and wastes, consumer products, drugs, and pesticides could act more effectively in the development of testing programs and interacting with other Government and private organizations if there were intramural staff members knowledgeable in each of these areas. At the present time, however, severe space limitations make it impossible to recruit established scientists who are adequately qualified for these positions. In spite of these restrictions, the contract program is expanding at a rapid rate. By the end of this fiscal year the Biological Models Segment will have approximately 24 contracts in effect; the Bioassay Section having responsibility for this Segment now consists of two permanent professionals, two staff fellows, and two visiting fellows (an additional staff fellow and an additional permanent professional will join late this year). The Bioassay Segment will have 20 contracts in effect with the Carcinogen Screening Section having five permanent professionals, one staff fellow, and one guest worker. New and expanded programs will be undertaken in future years; the success with which they will be coordinated will depend on the availability of qualified staff to perform this function. In order to effectively coordinate this research, it will be essential to have a "critical mass" of staff scientists who are thoroughly immersed in the type of research which they will manage, so that they will be aware of its problems, opportunities, and developments, and can effectively serve the function of examining the

plans and research of contract-supported organizations to be sure these represent the best interests of the Government.

Major activities in the intramural laboratories of the Branch are summarized in the following paragraphs:

CARCINOGEN SCREENING SECTION has devoted most of its effort to three problems, the activation of carcinogenic aromatic amines, the study of liver carcinogenesis by in vivo and in vitro systems, and the development and elucidation of experimental models for the induction of cancer of the colon.

Studies on the metabolism of the noncarcinogenic drug acetanilide, analogous to the carcinogen N-2-fluorenylacetamide, revealed common features to their metabolism. Specifically, metabolites were qualitatively and quantitatively different after a single acute dose compared to after chronic treatment. In addition to the known metabolites described in previous years, the new important discoveries were made that the so-called "water soluble metabolites" which are found with these agents and indeed with virtually all aromatic and heterocyclic chemicals and drugs, are of the nature of complex gluthathione, cysteine, and mercapturic acid derivatives. Their formation appears to hinge on the intermediate production either of an active N-hydroxy derivative or a ring epoxy compound. Further in vivo metabolism studies have demonstrated that aromatic nitro compounds are reduced to the amine via intermediary hydroxylamine stages. In many cases, however, the reaction from the nitro compound to the hydroxylamino step is much slower than the subsequent conversion to the amine. From these combined biochemical studies it can be concluded that some widely used nitroaryl derivatives, thus far not considered carcinogenic may well be so. On the basis of the biochemical results, carcinogenicity tests have been initiated to document this point. Phenobarbital enhanced N-hydroxylation of the carcinogen N-2-fluorenylacetamide but unexpectedly and curiously halved the binding of carcinogen to cellular molecules in the liver, like DNA. Coincidentally, phenobarbital treatment also decreased the carcinogenicity quite dramatically. The underlying mechanism is the induction of glucuronyl transferase by phenobarbital which rapidly conjugates the N-hydroxy derivative with glucuronic acid and hence renders it readily excretable through the kidney. This finding further documents indirectly our previous finding that the key liver carcinogen derived from this type of compound is the sulfate ester of the N-hydroxy derivative, which is in this instance formed in lower amounts because of the highly effective competing reaction leading to glucuronide. Just completed are experiments involving administration of the antioxidant butylated hydroxytoluene (BHT) which also reduced the carcinogenicity of N-2-fluorenylacetamide and of the N-hydroxy derivative. In this case, a similar increased formation of the glucuronic acid conjugates and hence competitively decreased sulfate ester formation was observed. Further developments of such competitive situations may lead to practical application in decreasing the carcinogenic potential of environmental agents.

Exposure of epithelial-like liver cells of rats, developed by unique methods in our laboratories, to a number of chemical carcinogens has led to transformation of these cell systems. Injection of the transformed cells into syngeneic hosts has reliably produced carcinoma, in contrast to many earlier

studies on in vitro transformation which have given sarcoma. Thus, our system provides a tool to study further the mechanism of carcinogenesis in vitro under controlled conditions and utilizing a system which produces cancer like that seen in man and animals. Further biochemical and histochemical studies of the liver cell system indicate that the amount of certain enzymes is different in a growing culture compared to a static non-multiplying culture. Further development of this system will be useful to gather information of phenomena of differentiation in relation to carcinogenesis. Potentially important results were achieved in additional exploration of in vitro systems in collaboration with Dr. R. Huebner and associates, utilizing a variety of chemical carcinogens in cell systems, infected or not, with the Rauscher leukemia virus. With the aromatic amines, agents of proven carcinogenicity in animals led to transformation while inactive isomers failed to do so. However, with a series of carcinogenic hydrocarbons and their noncarcinogenic analogs, the correlations were not as good. Further comprehensive investigations with a large number of agents of different chemical classes are currently underway. Some observations bearing on the underlying mechanisms lead to the preliminary conclusion that such cell systems infected with virus have a higher enzyme activity metabolizing carcinogens, specifically benzpyrene, compared to uninfected cells. This key finding may account for the synergism between chemical carcinogens and viral elements.

Human patients with liver cancer exhibit an abnormal globulin in their serum, alpha-fetoprotein. The presence of the protein is related more to liver cancer in certain populations in Africa compared to Europe or the United States. The reason for the differences is obscure. Yet it is important to understand the underlying mechanism, for this would throw light on specific factors connected with the etiology of the cancer and also serve as a useful diagnostic tool. In model studies a key discovery was made that several hepatocarcinogens lead to the early production of alpha-fetoprotein in rats. The presence of alpha-fetoprotein was thus noted with every agent studied. Interestingly the titer dropped even in the face of continuing treatment with carcinogen, until in the later stages it reappeared at the same time as overt cancer of the liver was found. In contrast to previous negative reports the important mycotoxin, aflatoxin, which is suspected to be one of the etiologic factors leading to liver cancer in man, does give rise to specific titers of alpha-fetoprotein.

Continuing model studies of the effect of diet in the production of colon cancer in mice and rats with 1,2-dimethylhydrazine or azoxymethane has shown that high protein and particularly high fat diets increase the toxicity of these carcinogens but not necessarily the carcinogenicity. Two enzymes in intestinal contents and in feces, which are capable of modifying carcinogen activity in the gut, exhibit diet-related variations in activity. The changes, however, relate more to the bulk of intestinal contents than to the specific enzyme activity.

BIOASSAY SECTION has continued to work with the technique for culturing skin cells of the mouse which have been used for experiments on carcinogenesis in previous years. Significant improvements have been made in the culture technique this year. Previous experiments had utilized cultures consisting of a mixture of epithelial cells in fibroblasts. It is now possible to eliminate

the vast majority of the fibroblasts for the culture and new procedures result in much-improved growth of the epithelial cells as well.

Investigations into the role of DNA synthesis in the process of carcinogenesis have continued. Previous experiments had suggested that DNA synthesis at the time the carcinogen is present or subsequently may be required for the initial stages of the malignant transformation to occur. Other experiments in this laboratory had indicated that DNA synthesis is not required for polycyclic hydrocarbon carcinogens to bind to DNA and that following binding, carcinogen-bound DNA can replicate and carry the carcinogen into the newly synthesized DNA on the parent strand. This suggested the possibility that the process of DNA synthesis may fix an abnormality into the hereditary apparatus by resulting either in a mutation or in an alteration in the pattern of differentiation. According to this hypothesis if repair mechanisms remove the bound carcinogen before DNA synthesis, no hereditary alteration would result. Therefore, considerable effort during the past year has been placed on getting background information on the biochemistry of the mouse skin cells which will permit studies on the repair of carcinogen binding in this system.

Since various carcinogens interact with different bases in DNA, the incorporation of thymidine, deoxycytidine, deoxyadenosine, and deoxyguanosine into DNA and RNA of cultured normal mouse skin cells was studied. After one hour in culture, more than 97% of both pyrimidine precursors incorporated into nucleic acids was found in DNA. However, with the purines, the majority of the tritium label was found in RNA. After twenty-four hours in culture, the percent of label in nucleic acids found in DNA had increased from 11% to 55% for deoxyadenosine and from 40% to 88% for deoxyguanosine. However, the specific activity of DNA labelled with purines remained low compared to that labelled with pyrimidine precursors.

Other experiments have investigated the ability of hydroxyurea to inhibit S-phase DNA synthesis without inhibiting repair synthesis in these cells and have examined the level of inhibition of DNA synthesis caused by various carcinogens.

Investigations elsewhere have demonstrated that enhancement of the rate of cell proliferation in mouse skin at the time that urethan is applied markedly enhances the carcinogenicity of this chemical. Preliminary results of experiments of the Bioassay Section confirm this observation but also extend it to indicate that enhanced cell proliferation 24 hours after the carcinogen has been applied, and at a time when only minimal levels of the carcinogen remain present in the animal, is equally effective in increasing the tumor yield induced by urethan.

Another major program within the Bioassay Section has been to develop animal models of the major forms of human childhood cancer and to study factors which affect the sensitivity of fetal and neo-natal tissues to chemical carcinogens. Phenomena under investigation include the differential sensitivity of fetal and adult mice of certain strains to the induction of pulmonary adenomas, the spontaneous regression of chemically induced lymphocytic lymphomas in Swiss mice, and the possible role of viruses in the induction of

subcutaneous sarcomas by injection of DEAE-DEXTRAN and other polycations. Evidence is beginning to accumulate that the differences in immune responsiveness may account for the susceptibility of fetal mice to the induction of pulmonary adenomas in certain strains in which adult members are resistant. Subcutaneous sarcomas induced by polycations were found to be negative for C-type viruses by both the XC test and by electron microscopy though the presence of murine leukemia virus can be identified in cells treated with IUdR.

New programs underway involve the search for techniques to induce the types of tumors seen in human children. The approaches used are combination of intrauterine or neonatal exposure to chemical carcinogens, manipulation of the animal to enhance the susceptibility to the carcinogen, and attempts to delay the maturation process of experimental animals in order to allow a longer period for tumor development.

The BIOASSAY SEGMENT has as its goals the evaluation of carcinogenic hazards in the environment, the development, refinement and application of methods related thereto, and the performance of additional studies on mechanisms of action of select agents as required to facilitate its overall goals. In addition to the Director and Manager of the Segment, there is an Advisory Group consisting of six scientists from various divisions of the National Cancer Institute, one from the Food and Drug Administration, and six non-Government scientists.

Attention has been given during the past year to standardizing the approach to bioassays of environmental chemicals so that results in one laboratory will be more comparable to those in others. Nine projects are now in effect using these standardized procedures for the bioassay of 148 compounds in approximately 80,000 animals. These chemicals include 37 pesticides, 31 drugs, 26 dyestuff intermediates and aromatic amines, 18 solvents, 24 chemical intermediates, and 6 chlorodibenzodioxans. Other contracts within the segment are designed to test industrial and environmental chemicals for carcinogenicity and co-carcinogenicity to the skin, the utilization of the pulmonary adenoma system in mice for identification of carcinogens, studies on carcinogenicity of fungal and plant products, and the effect of diet on chemical carcinogenesis.

Because of the expense in both time and money required for carcinogenesis-bioassay in vivo, the segment has been evaluating a variety of possible short-term tests to screen for carcinogenesis. In the coming year, it is anticipated that the more promising of these will undergo an additional evaluation to determine their general utility.

In order to facilitate the exchange of useful information, the Bioassay Segment is establishing contacts with a variety of national and international organizations such as the Food and Drug Administration, Environmental Protection Agency, Institute for Occupational Safety and Health, Pharmaceutical Manufacturing Association, Japanese National Institute of Health, Netherlands Public Health Institute, and International Agency for Research on Cancer.

BIOLOGICAL MODELS SEGMENT now consists of a Director and Manager, four NCI scientists, and seven scientists from academic and research institutions. During the past few years, the portions of the carcinogenesis contract program dealing with biological models has been devoted principally to the lung with only a few contracts dealing with all other organ systems programs on lung carcinogenesis and carcinogenesis of the colon and rectum have been established in other segments and the Biological Models Segment has been given the role of developing and studying models of other forms of cancer. The decision of the Segment has been to put particular emphasis on the following types of cancer: (1) pancreas, (2) endocrine related cancers such as prostate and breast, (3) kidney and urinary bladder, (4) tumors of childhood. Particular emphasis during the past year has been placed on initiating programs in carcinogenesis of the pancreas and prostate. It is anticipated that between seven and ten contracts will be initiated in these areas by the end of the fiscal year. In addition, two new contracts and one old contract deal with mammary carcinogenesis, one new contract on renal carcinogenesis and one on transplacental carcinogenesis are being established, and a previously established contract on liver carcinogenesis are continuing. A formerly established contract on the carcinogenesis of metallic compounds is now placing considerable emphasis on the roles of cadmium and zinc in the development of tumors of the prostate and testis. Other contracts within the Segment are designed to define the sensitivity of bioassay systems and to study the influence of combinations of chemicals on the carcinogenicity of individual components of the mixture.

Plans for the next fiscal year call for particular emphasis on development of programs for the urinary bladder and childhood tumors with an expansion of the other programs begun this year.

Serial No. NCI-4611
1. Experimental Pathology Branch
OASDC, Etiology
2. Bioassay Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Metabolic Events in the Early Stages of Chemical Carcinogenesis

Previous Serial Number: Same

Principal Investigator: Richard R. Bates, M.D.

Other Investigators: David L. Morgan

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	0.1
Other:	1.4

Project Description

Objectives:

The purpose of this project is to determine the importance of specific cellular metabolic activities in neoplastic transformation by chemical carcinogens and to develop new systems of chemical carcinogenesis which are particularly suitable for this type of investigation.

Methods Employed:

The principal tissue studied has been mouse skin. Studies in the intact mouse are designed to test the effect of various drugs and hormones on the induction of skin tumors by chemical carcinogens. Other studies are performed utilizing primary cell cultures of mouse epidermis. This culture technique was developed in the laboratory. The cell cultures are used for relatively long-term studies on malignant transformation induced by treatment of the primary cultures with chemical carcinogens. They are also used for short-term studies on the interaction of chemical carcinogens with cellular constituents and the effect of these interactions on cell function. Particular emphasis is placed on studying the interaction of carcinogens with DNA and the effect of this interaction on DNA function. Chromatographic techniques and ultracentrifugation are used to separate different types of DNA.

Major Findings:

Activity on this project has been reduced to a very low level during the past year because of the demand of administrative activities on the principal investigator and because of the lack of available support personnel. The major effort has been placed on improving the cell culture technique used in previous years. Formerly, these cultures consisted of mixtures of both epithelial cells and fibroblasts. During the past year enzymatic dissociation of epidermis from dermis has been utilized in order to eliminate the vast majority of fibroblasts from the cultures. It was also observed that the epidermal cells which were seeded had a much higher viability and proliferative rate if the underassociated epidermis was incubated overnight in cold balance salt solution before dissociating the cells.

Significance to Biomedical Research and the Program of the Institute:

The cell culture system developed in this laboratory has been very useful for the study of the early events in malignant transformation by chemical carcinogens. Previous investigations utilizing these cultures have indicated that the carcinogen dimethylbenzanthracene binds to non-replicating DNA at least as well as it does to replicating DNA. The carcinogen-bound DNA is then capable of further replication with the carcinogen being carried into the newly synthesized DNA on the parent strand. Since the malignant transformation is transmitted from one cell generation to the next it is important to study cellular molecules and organelles which carry phenotypic information from one cell generation to the next. DNA is a most important example of such a molecule. Thus it is essential that we learn more of how the structure and function of this molecule is affected by chemical carcinogens.

Proposed Course of Project:

During the coming fiscal year additional personnel will be available to work on this project. Effort will be concentrated on studying the in vitro transformation of the mouse skin cells by chemical carcinogens and on determining the role of altered DNA in the process of malignant transformation.

Honors and Awards

Director, Biological Models Segment, Carcinogenesis, NCI
Member, Lung Cancer Segment, Carcinogenesis, NCI
Member, Experimental Therapeutics Contract Review Committee, Chemotherapy, NCI
Member, Breast Cancer Virus Segment Working Group, Special Virus Cancer Program, NCI
Member of Working Cadre, National Prostate Cancer Project
Invited Speaker, American Cancer Society Conference on Cancer of the Pancreas
Member, Carcinogenesis Intramural Program Group, Etiology, NCI
Attendant, Meeting of the Food Protection Committee, National Academy of Sciences, National Research Council, January 1972
Member, Carcinogenesis Contract Program Management Group, Etiology, NCI

Serial No. NCI-4501
1. Experimental Pathology Branch
OASDC, Etiology
2. Bioassay Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Development of Animal Model of Adenocarcinoma of Prostate

Previous Serial Number: None

Principal Investigator: Richard R. Bates, M.D.

Other Investigators: Winston D. Edwards, Ph.D.

Cooperating Units: None

Man Years:
Total: 1.1
Professional: 0.5
Other: 0.6

Project Description

Objectives:

Although adenocarcinoma of the prostate is a major form of human cancer there are no experimental models for its induction by chemical or physical means. This project is designed to develop such a model and to study factors involved in its etiology and pathogenesis.

Methods Employed:

This project has recently been initiated and experiments are just getting underway. Plans call for a combination in vivo and in vitro exposure of the prostate of rodents to chemical carcinogens. Hormonal manipulations of the host or of organ culture medium will be performed to either increase or decrease the levels of various hormones to which the prostate is exposed. In addition, animals of varying ages from immature to old will be used.

Major Findings:

Long-term experiments are just getting underway. In order to determine ideal sites in which to transplant carcinogen-treated organ cultures, prostates have been transplanted in a variety of host sites. Transplantation beneath the kidney capsule was relatively unsuccessful. Transplantation into the spleen led to significant survival of the tissue but with some central necrosis. The most successful transplants were those into cleared mammary fat pads of the mouse. In this site the glands survived very well, and in

testosterone stimulated animals they showed marked cell proliferation and secretion.

Significance to Biomedical Research and the Program of the Institute:

An animal model of adenocarcinoma of the prostate is an essential tool for studying the etiology and pathogenesis of the human disease. Such studies are needed in order to develop methods for the prevention of this form of cancer.

Proposed Course of Project:

The project will continue for several years along the lines outlined under "Methods Employed".

Honors and Awards

See Project No. NCI-4611

Serial No. NCI-4502
1. Experimental Pathology Branch
OASDC, Etiology
2. Bioassay Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Role of DNA Replication and Repair in Chemical Carcinogenesis

Previous Serial Number: None

Principal Investigator: Henry Hennings, Ph.D.

Other Investigators: Sol del Ande Eaton, David Morgan, and Elroy Patterson

Cooperating Units: None

Man Years:
Total: 3.0
Professional: 1.0
Other: 2.0

Project Description

Objectives:

To examine the repair of DNA after carcinogen treatment; to examine the incorporation of tritiated deoxyribonucleosides and bases into DNA and RNA in normal and carcinogen-treated cell cultures; and to examine the importance of cell proliferation in skin carcinogenesis initiated by urethan.

Methods Employed:

In the biochemical experiments, cell cultures of fetal mouse skin grown in roller bottles are used for extraction of the nucleic acids by a standard phenol procedure. Further purification of the nucleic acids is accomplished by utilizing hydroxyapatite columns. In experiments in which BUdR-labeled DNA is to be separated from DNA of normal density, caesium chloride gradient centrifugation in a Beckman L2-65B ultracentrifuge is carried out. In experiments in which only the specific activity of total DNA or RNA is required, macromolecules are precipitated with cold perchloric acid, RNA is hydrolyzed with dilute alkali and DNA is hydrolyzed with hot perchloric acid.

In tumor induction experiments in susceptible female mice, skin tumors are initiated by the i.p. injection of 25mg of urethan and promoted by weekly application of 0.5% croton oil in acetone.

Major Findings:

Since various carcinogens interact with different bases in DNA, repair of the altered DNA after carcinogen interaction may be most profitably studied with precursors of all four DNA bases. Thus, incorporation of ^{3}H-thymidine, ^{3}H-deoxycytidine, ^{3}H-deoxyadenosine and ^{3}H-deoxyguanosine into DNA and RNA of normal cultured skin cells was studied. After 1 hour in culture, both of the pyrimidine precursors were incorporated fairly specifically into DNA; more than 97% of the activity incorporated into nucleic acids was found in DNA. However, with the purines, the majority of the tritium incorporated into nucleic acids was found in RNA. After 24 hours in culture, the per cent of the label in nucleic acids found in DNA had increased from 11% to 55% for ^{3}H-deoxyadenosine and from 40% to 88% for ^{3}H-deoxyguanosine, but the specific activity of DNA remained low compared to that found with the pyrimidine precursors. The specific activity of DNA after 24 hours of incorporation of either of the purine precursors was only 5-10% of that found with ^{3}H-deoxycytidine and 0.5-1% of that found with ^{3}H-thymidine. The purine deoxyribonucleosides already tested may be inadequate for studying DNA repair. Currently, other potential precursors of purines in DNA are being tested.

Hydroxyurea has been reported to inhibit S-phase DNA synthesis without affecting repair DNA synthesis. This inhibitor has been used in the study of repair in essentially non-dividing cells in culture, such as lymphocytes. An attempt was made to use hydroxyurea to study DNA repair in our rapidly-dividing cultures of mouse skin cells. The cultures were treated with a carcinogen (either β-propiolactone or 1,3-propanesultone) for 1 hour. The cells were washed and carcinogen-free medium containing 10^{-2}M hydroxyurea was added. After about 5 minutes, either ^{3}H-thymidine or ^{3}H-deoxycytidine was added and the incubation was continued for 1-12 hours. Under these conditions, it was hoped that the hydroxyurea would inhibit S-phase DNA synthesis to such an extent that incorporation of the precursor due to repair synthesis could be detected. However, the inhibition of precursor incorporation into DNA by hydroxyurea was only 95-98%, which did not allow unequivocal estimation of repair synthesis. It was calculated that if the extent of repair in skin cells is about the same as that found by others in lymphocytes, then an increase in specific activity of DNA of less than 50% could be expected in a rapidly-proliferating system.

A more profitable way to study repair of DNA after carcinogen treatment may be to label newly-synthesized DNA with both BUdR and a tritiated DNA precursor such as deoxycytidine or deoxyguanosine, then to separate heavy and light peaks of DNA by centrifugation to equilibration in a caesium chloride gradient, and determine whether tritium is associated with only the heavy peak (newly-synthesized) or with both peaks. Tritium associated with the light peak of DNA may represent incorporation of the precursor during DNA repair. A one-hour treatment with 5 x 10^{-4}Mβ-propiolactone, which reduced the specific activity of DNA to about 30% of the control value, is appropriate for looking at DNA repair in the 12 hours following removal of the carcinogen. The above approach, along with autoradiography of skin cells after carcinogen treatment and exposure to a tritiated DNA precursor to locate lightly-labeled non-S-phase cells, is now being pursued.

Stimulation of cell proliferation prior to initiation of mouse skin with urethan has been found to increase the yield of skin tumors in initiation-promotion experiments by five- to tenfold. In an attempt to determine whether cell proliferation at the time urethan is acting or some time after urethan action is important in the enhancement of initiation, a tumor induction experiment was set up in which a single application of croton oil (which increases the number of skin cells in DNA synthesis beginning at 12-15 hours, reaching a peak at 18-24 hours, and returning to normal by 4 - 5 days) was given at either 24 hours or 6 hours before or 1 hour after the initiating injection of urethan. Preliminary results after 14 weeks of promotion indicate that the 3 times at which the croton oil application was given with respect to the time of initiation were about equally effective in the enhancement of papilloma formation. This could indicate that cell proliferation 12 or more hours after urethan application (a time at which the level of urethan in the mouse is reduced to less than 10% of that found initially), and not cell proliferation at the time urethan is interacting with cellular macromolecules, is important in the enhancement of carcinogenesis. This conclusion is also suggested by the results in groups of mice which received a croton oil treatment 24 hours before urethan, but had DNA synthesis inhibited to less than 10% of the control level by hydroxyurea for about 9 hours from 1 hour before initiation till 8 hours after initiation. The number of tumors in these hydroxyurea-treated groups is as high or higher than that found in groups receiving just croton oil 24 hours before initiation. This experiment is being repeated and extended to include other croton oil treatment times.

Significance to Biomedical Research and the Program of the Institute:

If a carcinogen-induced alteration in DNA is necessary in the process of carcinogenesis, then the repair of the critical alteration should reduce subsequent tumor formation. Replication of the DNA before repair has taken place may result in a permanent non-repairable change in the base sequence of the newly-synthesized DNA. Conversely, errors in the repair process could also lead to an altered DNA. These experiments are designed to help define the roles of DNA replication and DNA repair in chemical carcinogenesis.

Proposed Course:

The proposed course of further experiments is indicated under "Major Findings".

Serial No. NCI-4687
1. Experimental Pathology Branch, OASDC, Etiology
2. Bioassay Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Experimental Models of Human Childhood Neoplasms

Previous Serial Number: Same

Principal Investigator: Jerry M. Rice, Ph.D.

Other Investigators: W. Graeme Cotton, D.V.S.M., Ph.D.; Sewa Ram Joshi, B.V.Sc., Ph.D.

Cooperating Units: None

Man Years:

Total:	3.0
Professional:	2.0
Other:	1.0

Project Description

Objectives:

To develop experimental animal models for the various morphological forms of human childhood cancer, and to utilize these models for the following purposes: to explore the differences in host response to tumors of the same organ systems induced in various animal species at different times during prenatal and postnatal life; to investigate the factors responsible for the greater sensitivity of fetal and neonatal animal tissues to chemical carcinogens, and the role of factors other than the carcinogens themselves in enhancing or suppressing chemical carcinogenesis in experimental animals during intrauterine life and infancy.

Methods Employed:

Carcinogenic organic compounds are synthesized, where necessary, using standard procedures. Animals of various species and strains are exposed to these compounds at various times during pre- and postnatal life, and the carcinogenic response in various organs is evaluated histologically. Short and long-term cell and organ culture techniques are used where necessary to explore the role of interferon and other host defense mechanisms, including cellular immunity, in resistance to carcinogenesis, and to study the co-carcinogenic effects of microbial and viral infections in chemically induced tumors. Surgical procedures, including skin grafting, adult thymectomy,

diffusion chamber implantation, etc., are used in evaluating antitumor defense mechanisms of the intact animal, and organic and biological chemical techniques are employed to determine the effects of modifications in chemical carcinogen structure and of metabolic factors on the response of host tissues to carcinogens as a function of age.

Major Findings:

Previously reported studies on the role of transplantation immunity in the response of different strains of mice to pulmonary carcinogenesis as a function of genetic background, and the importance of the abrogation of such effective antitumor responses in resistant animals by transplacental administration of a carcinogen at a sufficiently early stage in fetal life, are being pursued by Dr. Cotton. Cross reactions among pulmonary tumors induced transplacentally in resistant strains of mice, and between such tumors and the normal pulmonary tissue of susceptible strains, are being studied both by the utilization of in vivo techniques of tissue grafting (making use of the greater sensitivity of primary vs. secondary immune responses to suppression by X-irradiation) and by in vitro methods involving Cr^{51} release from target tumor cells. His initial findings support our original proposition that immune responsiveness is the key to the differential sensitivity of adult vs. fetal mice of genetically resistant strains. A major effort is underway to produce reagents for the exploration of cross-reactive antigenicity, by either in vitro cell-mediated immune responses demonstrated by Cr^{51} release, or by cross-reactive antibody, visualized by fluorescence techniques.

Further studies have been carried out on the phenomenon of spontaneous regression of chemically induced lymphocytic lymphomas in Swiss mice. We have found that this mechanism for suppression of tumor growth occurs not only in mice given a single injection of 1-ethyl-1-nitrosourea at five weeks of age, but also in animals in which this dose is divided, or divided and doubled (thereby increasing the lymphoma incidence from 50% to nearly 100% among treated animals), and also among animals which were treated by a single dose of the carcinogen in utero. Technical difficulties associated with efforts to prove that the spontaneous regression phenomenon is due to an immune response have not yet been overcome, and the cause of the regression phenomenon remains under investigation.

Subcutaneous sarcomas induced in mice by repeated local injections of DEAE-dextran, although negative for C-type viruses by both the SC test and by electron microscopy of cell cultures derived from these tumors, turn out often to be very readily inducible for murine leukemia virus when treated with IUdR. Tests for focus-forming activity in such cultures are now in progress; since most murine sarcoma viruses have been isolated by passage through other species, notably the rat, it will be of considerable interest if a sarcomagenic variant can be isolated from these cultures using the induction technique. It has been found that at least one other polycation, poly-D-lysine, which is structurally dissimilar to DEAE-dextran, is also capable of inducing sarcomas at the site of repeated subcutaneous injection in mice; this reinforces the contention that the sarcomagenic activity is a function only of the polycationic character of the injected material. Experiments are currently underway to determine whether the mouse is uniquely sensitive to such a mode of tumor induction, or whether

the rat and the golden hamster are also vulnerable. An in vivo experiment is also underway to investigate the possibility of synergism between DEAE-dextran, 1-ethyl-1-nitrosourea, and IUdR or BUdR in the induction of malignant lymphoma, local sarcomas, or other types of inducible neoplasms in mice.

A new program, initiated this year by Dr. Joshi, involves the chemical induction of nephroblastoma (Wilms' tumor) in the rat by oral administration of 7,12-dimethylbenz[a]anthracene (DMBA), followed or preceded by ovariectomy. Experiments underway include efforts to induce such tumors by transplacental means, using DMBA or other carcinogens alone or in combination; to investigate the role of congenital defects involving the urogenital system in predisposing rats towards susceptibility to this type of tumor; and to evaluate the role of vitamin A deficiency in predisposing towards sensitivity to chemical induction of these tumors. This association has been noted by epidemiologists who have investigated the association of Wilms' tumor with various malformations in man which are known to be caused by vitamin A deficiency in animals. These studies have been begun this year, and results from the first series have not yet been obtained.

Significance to Biomedical Research and the Program of the Institute:

Research for animal models of human childhood neoplasms should provide an insight into the types of causative agent and modes of exposures responsible for childhood cancer. It is to be expected that natural selection would tend to eliminate genotypes in the human population which predispose individuals to the development of fatal neoplasms before attaining reproductive age. Yet the incidence of tumors in childhood is high, and an increased understanding of the factors responsible for circumventing the innate resistance to oncogenesis in members of a genetically resistant population during prenatal and early postnatal life may be of importance. Studies of transplacental carcinogenesis in mice with one carcinogen have already provided some clues to ways in which genetic resistance to the development of neoplastic disease may be overriden.

Proposed Course:

The proposed course of this project is in two parts. The first envisions an expansion of research for models of human tumors characteristically appearing in childhood. There is at present no model of the embryonal tumors often seen in children, although Wilms' tumor currently appears vulnerable. The advantage to the Institute of securing models of such neoplasms consists in the concomitant acquisition of knowledge concerning at least one method by which such tumors can be induced, and therefore a possible insight into the mechanisms of genesis of these tumors in human beings. A special area of investigation of interest is carcinogenesis in utero, by transplacental exposure to carcinogens circulating in the mother's blood stream. The high incidence of tumors in childhood, including occasional congenital neoplasms, suggests that transplacental exposure to chemicals may be an important route for human exposure to carcinogens. The experimental models which are currently available, however, have been incompletely explored. The only really effective transplacental carcinogen known at present is ethylnitrosourea. Furthermore, its effects in mice and rats, although including tumors of the central nervous system and of the hematopoietic tissues, have included few embryonal tumors, and oncogenicity

has not been demonstrated in mice exposed during early fetal or embryonic life. It is not known whether this represents a failure of the compound to reach susceptible cells, or a resistance to such cells to chemical neoplastic transformation, or whether this leads inevitably to tumors of such bizarre characteristics that they are eliminated by host defense mechanisms before they attain significant size. Further exploration of these aspects of carcinogenesis will require a contract-operated facility for the housing of animals of several species in significant numbers, and additional personnel with experience in embryology and pathology who can assume a significant amount of responsibility for these programs.

A second factor related to the first is the matter of increased susceptibility to carcinogenesis during perinatal life as a result of the abrogation of defense mechanisms available to the adult. Approaches to these problems have already been developed, as indicated in the "Findings" section above. These should be pursued, until the phenomena are fully understood. The participation of expert collaborators in fields of virology, immunology, and other areas should be sought as necessary to achieve this goal.

Another factor suggested by present studies on transplacental carcinogenesis is the question of whether tumors which are characteristically those of adulthood or even of old age may arise as a result of exposure to carcinogens during fetal or neonatal life. It is certainly true that in the mouse a higher percentage of tumors which are morphologically of the adult type are induced in animals exposed transplacentally or neonatally to ethylnitrosourea. The doses of carcinogen used in previous studies have generally been large enough to give rise to a high multiplicity of tumors. It is reasonable to expect that if this dose is lowered, and if it is administered at the stage in fetal life when the susceptibility to, e.g., pulmonary carcinogenesis, is greatest, a significant proportion of animals will develop a solitary, large, rapidly growing and highly malignant tumor which would bear a much closer resemblance to the human situation than any studies yet carried out.

Honors and Awards

Lecturer, NIH Workshop on Leukemias and Lymphomas of Animals and Man on May 14, 1971. Presented a paper entitled "Spontaneous regression of ethyl-nitrosourea-induced malignant lymphoma in Swiss mice".

Lecturer, Meeting on Transplacental Carcinogenesis sponsored by the International Agency for Research on Cancer, Hannover, Germany, October 5-7, 1971. Presented a paper entitled "Biological behavior of transplacentally induced tumors in mice".

Lecturer, FDA-NCI Workshop on Criteria for the Evaluation of Carcinogenic Hazards of Drugs, Rockville, Maryland, December 6-7, 1971. Presented a paper entitled "Intrauterine and perinatal carcinogenesis".

Publications

Rice, J.M.: Transplacental carcinogenesis in mice by 1-ethyl-1-nitrosourea. Ann. NY Acad. Sci. 163: 813-827, 1969

Rice, J.M., Turner, W., Chirigos, M.M., and Rice, N.R.: Enhancement by poly-D-lysine of poly I:C-induced interferon production in mice. Appl. Microbiol. 19: 867-869, 1970

Rice, J.M., Turner, W., Chirigos, M.A., and Spahn, G.: Dose responsiveness and variation among inbred strains of mice in production of interferon after treatment with poly(I)·poly(C) [poly-D-lysine] complexes. Appl. Microbiol. 22: 380-386, 1971

Rice, J.M., and Davidson, J.K.: Spontaneous regression of chemically induced malignant lymphoma in Swiss mice. Cancer Res. 31: 2008-2017, 1971

Catalano, L.W., London, W.T., Rice, J.M., and Sever, J.L.: Prophylactic and therapeutic use of poly(I)·poly(C) [poly-D-lysine] against herpesvirus encephalitis in mice. Proc. Soc. Exp. Biol. Med. (in press)

Rice, J.M., and Madison, R.M.: Subcutaneous injections of vaccine adjuvant DEAE-dextran induce local sarcomas in mice. Nature New Biology 236: 28, 1972

Serial No. NCI-4617
1. Experimental Pathology Branch, OASDC, Etiology
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Carcinogen Screening Operations

Previous Serial Number: Same

Principal Investigators: J. H. Weisburger, Ph.D. and E. K. Weisburger, Ph.D.

Other Investigators: L. A. Poirier, Ph.D. and J. M. Sontag, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.8
Professional:	1.4
Other:	0.4

Project Description

Objectives:

Management responsibilities are assumed on the design, performance and evaluation of studies in contract laboratories on the chronic effect and of the carcinogenicity of chemicals or of mixtures of chemicals. The aim of these investigations is to gather information on hazards involved in handling certain chemicals or drugs, and also on the mechanisms underlying joint effects in mixtures. Of particular interest are such mixtures which tend to give increased or decreased overall carcinogenic effect. Of concern also is the methodology of carcinogen screening, with emphasis on improvement in sensitivity, speed and economy. Short-term assays such as those based on mutagenesis are under development, taking into account the need to secure biochemical activation of most environmental agents.

Methods Employed:

Attempts are made to secure information on chemicals representing the greatest hazard to the greatest number of people. Chemicals and drugs are rated as to priority on this basis as well as other criteria such as relationship to known carcinogens, epidemiologic observations, indications of specific toxicity and related factors. Standard protocols (see Weisburger

and Weisburger, Methods in Cancer Research, Vol. 1, 1967) as well as newly designed protocols attempting to increase the sensitivity and speed of such tests are utilized. For example, recent developments on in vitro transformations by chemical carcinogens, of select cell systems, of the newer relationships between mutagenesis and carcinogenesis, and similar advances in the fundamental underlying sciences suggests that a battery of short-term preliminary screens can be developed based on these basic areas. Exploratory meetings with relevant national and international organizations in industry, in government, and in university environments are setting the stage for a concerted developmental effort in this area.

Major Findings:

In the current year several of the long-term experiments in contract laboratories are nearing the data generation stage, even though in some cases statistical evaluation is yet to be done. A number of the chemicals including drugs, industrial chemicals, pesticides, and environmental agents have been found to lead to cancer in test systems involving mice or rats.

1. Drugs. Additional data are available from a study of drugs used in cancer chemotherapy. Last year Melphalan, chlorambucil, uracil mustard, procarbazine, dimethyltriazinoimidazole carboxamide, BCNU and CCNU were reported as active in a preliminary way. Increased tumor incidence was now found in animals treated with 6-mercaptopurine, guanazole, actinomycin D, cytoxan, azathiaprine, o-melphalan, and the mixture of actinomycin D, chlorambucil, and methotrexate. Most of the tumors, especially in mice, were those in the hematopoietic system, namely leukemia, lymphosarcomas, reticulum cell sarcomas and plasmacytomas. Lung tumors in mice, and breast cancer in rats was also observed. It is hoped that these complex and large scale experiments will come to a definitive data evaluation point within the next year. At that time the results obtained should assist the clinic in selecting those drugs which exhibit the lowest long-term risk and at the same time provide the highest therapeutic efficiency.

2. Industrial chemicals. Preliminary information from tests in mice and rats indicates that 2,4,6-trimethylaniline, 2,4,5-trimethylaniline, 2,4-diaminotoluene, 2,4-xylidine, o-toluidine, 4-chloro-o-toluidine, 4,4'-methylene-bis-2-chloroaniline, benzoguanamine, 1-chloro-4-nitrobenzene, 1-chloro-2-nitrobenzene, 2,5-dimethoxy-4'-aminostilbene are carcinogenic, either in mice or in rats, or both.

Preliminary information indicates that the following are not active in male and female rats: semicarbazide, thiosemicarbazide, ethylene carbonate, sodium azide, and sodium sulfhydrate.

3. Pesticides. Ethylene thiourea, present as a contaminant in a number of thiocarbamic acid pesticides, and which led to induction of hepatoma in mice, yielded thyroid cancer in a high proportion of rats treated. On the other hand, a number of agents which lead to tumors in mice in an earlier study

so far have given no indication of carcinogenicity in rats. They are dithiooxamide, bis(2-chloroethyl)ether, Avadex, and bis(2-hydroxyethyl)dithiocarbamic acid (K salt). Gross autopsy results indicate that Mirex led to nodular livers in rats but the nature of the lesion requires histopathological study.

4. Natural products. Dose response studies on aflatoxin B_1 and a comparison of aflatoxin B_1 and the metabolite aflatoxin M_1 are in progress. The major metabolite of aflatoxin B_1, namely the O-desmethyl derivative, aflatoxin P_1, is being produced to study toxicity and carcinogenic activity. Studies are currently in progress to isolate products from about 30 fungi isolated from Asian foodstuffs, in particular those collected in a now discontinued program operated under contract in Bangkok, Thailand. Preliminary information indicated hepatotoxic activity associated with each of the fungi which appeared different from aflatoxin B_1. Thus, work-up of this material may uncover new chemical mycotoxins, of possible relevance to disease in man.

5. Nitrosamines - are they from secondary amines and nitrite in vivo? Regular workshops organized by FDA and other interested parties have been attended in order to contribute to and participate in discussions and experimentation on this key question. Newer data suggest that nitrosamine formation can occur under in vivo conditions in animal systems. The reality of such reactions in the human environment remains to be documented.

6. Artificial sweeteners. This office continues to act as a focal point for information exchange on the safety factors resulting from the use of artificial sweeteners. A number of studies dealing specifically with cyclamates and saccharin and related materials are reaching the data generation point this year. One study dealing with cyclohexylamine, a metabolite of cyclamate, has indicated that it was not carcinogenic.

Significance to Biomedical Research and the Program of the Institute:

Many factors in the environment have not yet been evaluated for chronic toxicity and possible carcinogenicity. It is important to secure such information after deliberate and judicious setting of priorities. Cancer in man has been observed in the past as a result of exposure to chemicals subsequently demonstrated to be carcinogenic. The aim of this program is to forestall such unintentional exposures of man which would result in neoplastic disease in the future.

Proposed Course of Project:

Continuing efforts are required 1. to assess relative priorities of materials to be tested, 2. to undertake studies on variations in the protocols to simplify or to develop novel test techniques which are more sensitive, more specific and more economic, 3. and to apply such tests to identify carcinogenic hazards in the environment.

In view of the establishment of the Bioassay Segment, Carcinogenesis, Etiology, which essentially has assumed the enlarged responsibilities for carcinogen screening operations, this will be the last specific report under the aegis of the Carcinogen Screening Section. In the future, the annual report of the Bioassay Segment will deal with results obtained in operations covered by its responsibilities.

Honors and Awards

J. H. Weisburger:

Associate Editor, CANCER RESEARCH, 1969-1972

Member, Editorial Committee, CANCER RESEARCH, American Association for Cancer Research

Member, Editorial Board, FOOD AND COSMETICS TOXICOLOGY

Member, Editorial Board, CHEMICO-BIOLOGICAL INTERACTIONS

Member, Editorial Board, XENOBIOTICA

Invited Lecture: J. H. Weisburger: Causes and Prevention of Cancer. Alpha Chi Sigma Fraternity, Washington, D. C., January 15, 1971.

Invited Lecture: J. H. Weisburger: On the Etiology of Cancer and the Mechanisms of Carcinogenesis, with Emphasis on Aromatic Amines. Symposium, Food and Drug Directorate, Ottawa, Ontario, Canada, June 11, 1971.

Invited Lecture: J. H. Weisburger: Current Test Procedures for Assessment of Carcinogenicity. Symposium, The American Society for Pharmacology and Experimental Therapeutics, Burlington, Vermont, August 22-26, 1971.

Invited Lecture: J. H. Weisburger: Biochemical Pharmacology, Molecular Biology, and the Elements of Cancer Causation and Prevention. Symposium on the Role of Basic Research on the Current Status of the Cancer Problem. American Chemical Society, Washington, D. C., September 15, 1971.

Invited Lecture: J. H. Weisburger: The Mechanisms of Action of Environmental Carcinogens. Department of Biological Sciences, University of Delaware, Newark, Delaware, October 1, 1971.

Invited Lecture: J. H. Weisburger: Model Studies on the Etiology of Colon Cancer. Princess Takamatsu Cancer Symposium, Tokyo, Japan, November 16-18, 1971. Also Session Chairman.

Invited Lectures: J. H. Weisburger: a) Current Concepts in Colon Carcinogenesis; b) Chemical Carcinogenesis and the Etiology of Cancer; c) Evidence that Sulfate Esters are the Ultimate Carcinogens Involved in Hepatocarcinogenesis with N-2-Fluorenylacetamide. National Cancer Center Research Institute, Tokyo; Nara Medical College, Japan; University of Osaka, Japan; Cancer Institute, Tokyo, and University of Hawaii, Honolulu. November 1971.

Invited Lecture: J. H. Weisburger: Current Status of Safety Evaluation of Artificial Sweeteners. Calorie Control Council Meeting, Washington, D. C., November 30, 1971.

Invited Lecture: J. H. Weisburger: Metabolism of Chemical Carcinogens in Various Species. Workshop on "Criteria for the Evaluation of Carcinogenic Hazards of Drugs." Food and Drug Administration, Rockville, Maryland, December 6 and 7, 1971.

Invited Lecture: J. H. Weisburger: Chemical Carcinogenesis in Japan: Report on 2nd Princess Takamatsu Cancer Symposium, and on In Vitro Studies with Liver Cells, Including Movie from Professor Katsuta's Laboratory, University of Tokyo. Experimental Pathology Branch Seminar, January 6, 1972.

Invited Lecture: J. H. Weisburger: Current Concepts of Cancer Causation. Food and Drug Administration, Rockville, Maryland, January 21, 1972.

Invited Lecture: J. H. Weisburger: Current Concepts of Cancer Causation. Virginia Commonwealth University, Richmond, Virginia, March 10, 1972.

Invited Lecture: J. H. Weisburger: Diet and Experimental Models of Colon Cancer. New York Cancer Society, New York, March 14, 1972.

Invited Lecture: J. H. Weisburger: Chemical Carcinogens and Their Mode of Action in Colonic Neoplasia. American Proctologic Society, Symposium, New York, New York, June 12-15, 1972.

Invited Lecture: J. H. Weisburger: Carcinogenesis and its Importance in Clinical Cancer. American College of Physicians Postgraduate Course, Perspectives in Cancer Medicine, Buffalo, New York, June 26-29, 1972.

J. H. Weisburger: Organizer and Chairman, Symposium on Frontiers of Cancer Research, University of Cincinnati, Ohio, June 8, 1971.

J. H. Weisburger: Symposium speaker and Chairman of Session on Mutagenesis and Carcinogenesis. Society of Toxicology meeting, Williamsburg, Virginia, March 5-9, 1972.

J. H. Weisburger: Group Chairman, National Cancer Plan, 1971-1972.

Publications

Prejean, J. D., Griswold, D. P., and Weisburger, J. H.: Transplantation of allogeneic tumors in rats and mice treated with azathioprine, prednisone and antilymphocyte serum. Proc. Soc. Exptl. Biol. Med. 139: 1425-1428, 1972.

Ulland, B., Finkelstein, M., Weisburger, E. K., Rice, J. M., and Weisburger, J. H.: New carcinogenic industrial chemicals. Propylene imine and propane sultone. Nature 230: 460-461, 1971.

Weisburger, E. K.: Testing of new compounds for long-term toxicity. J. Soc. Cosmet. Chem. 22: 825-838, 1971.

Weisburger, J. H.: Chemical Carcinogenesis. In Holland, J. F. and Frei, E. (Eds.): Cancer Medicine, Lea and Febiger, Philadelphia (in press).

Weisburger, J. H. and Weisburger, E. K.: Chemical carcinogens, cancer research, and the prevention of cancer. In Lettre, H. and Wagner, G. (Eds.): Aktuelle Probleme aus dem Gebiet der Cancerologie. III. Drittes Heidelberger Symposion (Vol. 9). Berlin/Heidelberger/New York, Springer-Verlag, 1971, pp. 3-28.

Weisburger, J. H. and Williams, G. M.: Cancer tests. The relation of cancer induction and genetic damage. Proc. Symposium on Evaluation of Genetic Risks of Environmental Pollutants, Stockholm, Sweden (in press).

Serial No. NCI-4618
1. Experimental Pathology Branch, OASDC, Etiology
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mode of Action of Chemical Carcinogens -- Endogenous and Exogenous Factors in Chemical Carcinogenesis

Previous Serial Number: Same

Principal Investigator: J. H. Weisburger, Ph.D.

Other Investigators: R. S. Yamamoto, Sc.D., E. K. Weisburger, Ph.D., R. Kroes, D.V.M., Ph.D., and J. M. Sontag, Ph.D.

Cooperating Units: Dr. Russell M. Madison, Microbiological Associates
Dr. Borge M. Ulland, Bionetics Research Laboratories, Inc.
Drs. J. D. Prejean and D. P. Griswold, Southern Research Institute

Man Years:

Total:	3.6
Professional:	0.9
Other:	2.7

Project Description

Objectives:

Investigations to discover the intimate factors involved in the initiation and development of neoplasia are explored by means of animal experiments in various species. The studies utilized a number of chemical carcinogens administered to animals under varying conditions of diet, endocrine situation or immunological status so as to gain insight into controlling mechanisms in the initiation and development of chemically induced tumors. Further efforts deal in the development of systems which lead to the prevention of the processes of chemical carcinogenesis in a study of the mechanisms related thereto.

Methods Employed:

Chemical carcinogens or unknown agents to be evaluated for carcinogenicity are administered by a number of routes to several strains of rats or mice on specific dietary regimens. Additionally, certain chemicals with specifically

tailored structures or properties are given together with chemical carcinogens to determine whether tumor induction can be delayed or inhibited. Host factors such as the endocrine or immunologic status are being investigated in relation to the effectiveness of tumor formation. After judiciously selected treatment and observation periods, autopsies of the experimental animals are performed. Upon histologic processing of tissues, the results in experimental systems are carefully evaluated in relation to control groups.

Major Findings:

1. On the mode of action of carcinogenic aromatic amines. Our group has reported earlier the important and unique discovery that the sulfate ester of N-hydroxy-N-2-fluorenylacetamide is the significant ultimate carcinogen for the induction of liver cancer with the parent compound N-2-fluorenylacetamide. Documentation for this fact rested on the restoration of carcinogenicity by dietary sulfate ion under conditions where the action of the carcinogen was inhibited with acetanilide. The rationale was based on a depletion of available sulfate by p-hydroxyacetanilide, the chief metabolite of acetanilide. It seemed, therefore, that p-hydroxyacetanilide should be more effective than acetanilide.

In the current year it was found, however, that the reverse was true: Acetanilide was more effective than the p-hydroxy metabolite. Furthermore, addition of sulfate to the diet increased the inhibition rather than restored carcinogenicity when p-hydroxyacetanilide was used. Thus, the situation is more complex than was thought earlier.

The combined data, namely inhibition of carcinogenicity of N-2-fluorenylacetamide or of the N-hydroxy derivative by acetanilide, increased carcinogenicity in the presence of excess dietary sulfate ion, but little inhibition with p-hydroxyacetanilide can be interpreted as follows. The key inhibitory activity rests on the demonstrated interference with N-hydroxylation of N-2-fluorenylacetamide. The lesser inhibitory activity of p-hydroxyacetanilide derives from the fact that it does not inhibit N-hydroxylation. Administration of the N-hydroxy derivative leads to biliary excretion and we have shown some years ago that this metabolite is converted back to N-2-fluorenylacetamide by the intestinal flora. This compound is resorbed from the lower gut, and converted to the N-hydroxy compound, the active carcinogen in the liver since the p-hydroxyacetanilide fails to inhibit this reaction whereas acetanilide does. Acetanilide is more effective than p-hydroxyacetanilide because it blocks both activation steps, and the latter compounds only one.

Thus far the involvement of the sulfate ester of N-hydroxyfluorenylacetamide was restricted to liver carcinogenesis in the male rat. In the current year efforts were also centered on extending this finding to other systems as regards target sites, species, and different aromatic amine carcinogens. It was found that in female Charles River rats acetanilide inhibited breast cancer induction by fluorenylacetamide but p-hydroxyacetanilide failed to inhibit mammary tumor formation by N-hydroxy-N-2-fluorenylacetamide. Interestingly,

liver cyst formation by either carcinogen (no liver tumors were found in our tests) was inhibited by acetanilide and p-hydroxyacetanilide. In male and female mice fed N-2-fluorenylacetamide, acetanilide, or p-hydroxyacetanilide lowered liver tumor formation, but p-hydroxyacetanilide failed to affect liver tumor formation due to N-hydroxy-N-2-fluorenylacetamide. Similar studies in hamsters were just completed and results are being evaluated. Acetanilide also inhibited liver tumor formation and the appearance of precancerous lesions in rats fed carcinogenic azo dye, 3'-methyl-4-dimethylaminoazobenzene. Studies with the more potent liver carcinogen 6 -dimethylaminophenylazoquinoline in the same system are not quite complete.

In contrast to the inhibition of breast tumor induction in rats by acetanilide when the carcinogen was N-2-fluorenylacetamide or the N-hydroxy derivative, acetanilide failed to inhibit breast tumor formation with benzidine or 2-anthramine, given as 10 doses in oil by the so-called "Huggins technique." Also, skin tumor formation in rats after cutaneous application of 2-anthramine was not inhibited by the simultaneous feeding of acetanilide.

Studies are still incomplete on the question whether acetanilide would inhibit ear duct tumor induction with N,N-dimethyl-4-aminostilbene, kidney tumor induction with 4'-fluoro-4-aminobiphenyl, or colon cancer induction by 2',3-dimethyl-4-aminobiphenyl. It is hoped that a consideration of all the tests in this area, completed and still in course, will lead to a reasonably clear picture on the need for 1) N-hydroxylation by aromatic amines, 2) further esterification of the N-hydroxy derivative in relation to target organs, species, and structure of the aromatic amine.

2. Effect of antioxidant on the carcinogenicity of N-2-fluorenylacetamide or N-hydroxy-N-2-fluorenylacetamide. We reported last year that vitamin C or diphenyl-p-phenylenediamine failed to inhibit liver tumor formation by the two carcinogens mentioned in the title. Experiments are now completed showing that another widely used antioxidant, butylated hydroxytoluene (BHT) did very significantly reduce liver cancer induction by these agents. It has already been found that the antioxidant 1) was required in a large molar excess over the carcinogen, 2) failed to affect sulfate metabolism, and 3) dietary supplements of sulfate failed to overcome the inhibition, 4) the livers of BHT-fed rats were appreciably enlarged at high dose levels of BHT but not at lower dose levels. This suggested that BHT, like phenobarbital, might operate via enzyme induction. Experiments supporting this view are detailed in NCI-4619.

3. The African White-tailed rat - *Mystromys albicaudatus* - is not a good species for carcinogen bioassay. The experiment mentioned in a preliminary way last year in which this rodent species, reported to have a low spontaneous tumor incidence, and thus of considerable interest for carcinogen testing, responded only slowly to a regimen of 40 to 100 ppm diethylnitrosamine in drinking water. The histologic study now shows that the key lesions induced were hepatocellular carcinoma, and some liver cyst formation. The forestomach was affected in some cases; the lesion appears to be dysplasia.

Only the Chinese hamster exhibits similar lesions in the forestomach when treated with diethylnitrosamine. The overall impression from these tests is that the species fails to meet the criteria of easy management, ready reproductive capability, and above all sensitivity to a typical carcinogen to be useful in such tests.

Significance to Biomedical Research and the Program of the Institute:

The prevention and cure of neoplasia hinges on an understanding of the intimate factors involved in the pathogenesis of cancer. The research performed within the framework of this report aims to produce data which will permit the comprehension of the underlying mechanisms involved in chemical carcinogenesis.

Proposed Course of Project:

A number of the tests for chronic toxicity and carcinogenicity presently underway or planned to go on stream shortly will require considerable effort for several years. Additional emphasis will be placed to find systems of fundamental value which will give insight into methods for inhibiting the carcinogenic process and to understand the various steps leading to such inhibitions. Thus studies will be variations in endogenous factors as well as select exogenous materials.

Publications

Deckers, C., Glass, R. M., Grantham, P. H., Yamamoto, R. S., and Weisburger, J. H.: A comparative study of the proteins of rat plasma, liver and hepatoma by agarose immunoelectrophoresis. Brit. J. Cancer (in press).

Yamamoto, R. S., Frankel, H. H., Williams, G. M., and Weisburger, J. H.: 8-Hydroxyquinoline: Chronic toxicity and inhibitory effect on the carcinogenicity of N-2-fluorenylacetamide. Toxicol. Appl. Pharmac. 19: 687-698, 1971.

Yamamoto, R. S., Kroes, R., and Weisburger, J. H.: Carcinogenicity of diethylnitrosamine in *Mystromys albicaudatus* (African White-tailed rat). Proc. Soc. Exptl. Biol. Med. (in press).

Yamamoto, R. S., Weisburger, J. H., and Weisburger, E. K.: Controlling factors in urethan carcinogenesis in mice: Effect of enzyme inducers and metabolic inhibitors. Cancer Res. 31: 483-486, 1971.

Serial No. NCI-4619
1. Experimental Pathology Branch, OASDC, Etiology
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mode of Action of Chemical Carcinogens -- Studies on the Metabolism of Chemical Carcinogens

Previous Serial Number: Same

Principal Investigator: J. H. Weisburger, Ph.D.

Other Investigators: P. H. Grantham, M. S., E. K. Weisburger, Ph.D., N. B. Giao, D.V.M., Ph.D., L. A. Poirier, Ph.D., and J. Idoine, M. S.

Cooperating Units: None

Man Years:

Total:	4.6
Professional:	2.4
Other:	2.2

Project Description

Objectives:

The aim of this research is to gather data relevant to the etiology of neoplasia at the molecular level. To this end chemical carcinogens, especially those of the aromatic amine type, are being utilized. Their metabolism and interaction with host tissues and specific targets are studied. Simplified yet realistic model systems are devised in order to gain an understanding of the fundamental events operating in chemical carcinogenesis. Specific inhibitors, and accelerators of the carcinogenic process are applied as tools to develop information on and permit discrimination between biochemical events directly involved in the neoplastic change and those representing other reactions.

Methods Employed:

Biochemical and pharmacological techniques are applied to determine the metabolism of N-hydroxy-N-2-fluorenylacetamide and related compounds in various animal species. This includes isolation and purification of enzyme systems concerned with certain metabolic steps.

Procedures are developed and applied for the separation, purification and analysis of macromolecular constituents such as DNA, RNA and proteins from tissues of animals treated with chemical carcinogens and from control animals.

The metabolism of chemical carcinogens and related compounds, and interaction with host targets is examined in vivo and in vitro in the presence of various chemicals or antibiotics which affect the carcinogenic process.

Major Findings:

1. Water-soluble metabolites of aromatic amines, specifically acetanilide, N-2-fluorenylacetamide and N-hydroxy-N-2-fluorenylacetamide. Efforts underway for some years to identify water-soluble metabolites found as biotransformation products of many lipophilic agents, normally water-insoluble but especially the chemicals named in the title, are slowly yielding to investigation. There is now preliminary yet fairly convincing evidence that one of the water-soluble metabolites derived from acute doses as well as after subacute intake (see last year's report) of acetanilide is the N-acetylcysteine derivative attached to the 3-position of p-hydroxyacetanilide, hence a typical mercapturic acid. Confirmation of the structure would suggest that its mode of formation involves an intermediate 3,4-epoxide of acetanilide. Another as yet incompletely characterized metabolite is one with mercapturic acid residue, probably in the 2-position of acetanilide. If this structure is real, the mode of formation would be quite different from the previously discussed metabolite, and most likely would hinge on an intermediary formation of a hydroxylamino derivative. However, despite extensive efforts analyses of urine of rats given single doses or chronic doses of acetanilide have yet to reveal the presence of such a hydroxylamino derivative. Of course, its potential reactivity may preclude its excretion in urine, and thus account for the formation of a mercapturic acid derivative. There is also good evidence that similar mercapturic acid derivatives constitute the bulk of the so-called water-soluble metabolites of carcinogenic N-2-fluorenylacetamide and of the N-hydroxy derivative but additional efforts now underway are required to completely secure such structures.

In connection with these investigations a gas-liquid chromatographic system was set up and standardized so that metabolites of acetanilide, N-2-fluorenylacetamide and of benzo(a)pyrene could be determined quantitatively with high accuracy and sensitivity.

2. Effect of the antioxidant butylated hydroxytoluene (BHT) on the metabolism of N-2-fluorenylacetamide and N-hydroxy-N-2-fluorenylacetamide in male and female rats. As described in NCI-4618, BHT decreased very appreciably the carcinogenicity of the agents mentioned in the title in male and female rats, on the liver and the breast, respectively. The inhibition could not be traced to decreased levels of available sulfate due to BHT, or to the antioxidant properties of BHT. Because of considerable increases in liver to body weight ratios in animals on BHT, investigations were carried out on the possibility that BHT might affect the metabolism of the carcinogen.

It was found that animals prefed BHT for 4 weeks had much lower blood levels of radioactivity from carcinogen and higher urinary metabolites with either N-2-fluorenylacetamide or the N-hydroxy derivative. Liver levels in animals given BHT were 1/2 or less, as was the amount of carcinogen specifically bound to liver DNA. The main metabolite accounting for the increased urinary excretion was the glucosiduronic acid fraction, which was more than doubled in male and in female rats. Thus, the mode of action of BHT in inhibiting carcinogenicity by these agents would appear to be increased formation and excretion of the glucuronic acid conjugates, so that less of the active intermediate can be formed to elicit the carcinogenic change. Indirectly, this experiment thus provides further support for the concept derived from our other studies, that the key intermediate for liver carcinogenesis is the sulfate ester of N-hydroxy-N-2-fluorenylacetamide.

3. Biochemical reduction of nitroaryl compounds in relation to carcinogenicity. The concept that aromatic amines are carcinogenic in animals and in man because they undergo biochemical N-hydroxylation is now well established. The key intermediary hydroxylamino derivative can also be formed by reduction of nitroaryl compounds. Presumptive evidence suggested that the reduction of nitro compounds might not be as stereospecific as the oxidation of the corresponding aromatic amine. Therefore, nitroaryl compounds might exhibit a more general carcinogenic activity and hence constitute broader carcinogenic hazards than would be the corresponding aromatic amines. For example, whereas 2-naphthylamine and 2-fluoreneamine are active carcinogens, the corresponding 1-naphthylamine or 1-fluoreneamine derivatives are not carcinogenic. Others have demonstrated, however, that the related 1-naphthylhydroxylamine or 1-fluorenylhydroxylamine derivatives are highly carcinogenic in animals. Because if was heretofore widely assumed that 1-nitronaphthalene and similar compounds are innocuous, they are extensively used as chemical intermediates.

The biochemical reduction of select nitroaryl derivatives under in vitro conditions with rat or mouse liver microsomes, the cell sap, or a mixture of the two as the post-mitochondrial fraction was studied in respect to nitroreductase activity. In addition, because certain nitroaryl compounds can cause tumors in the forestomach of rodents, a few tests were conducted with fractions from this organ. In a preliminary way also, contents of cecum of rats were used to test the idea that bacterial systems in the gut can effect reduction of such nitro compounds. By a variety of techniques,

including gas-liquid chromatography, thin layer chromatography, and colorimetric techniques, it was established that various aryl nitro compounds, in particular 1-nitronaphthalene, 2-nitronaphthalene and 4-nitrobiphenyl were reduced by enzymes from rat liver fractions to the corresponding amines. The reaction requires cofactors such as flavine mononucleotide (FMN) and NADPH. Despite serious efforts, however, no direct evidence for the formation of an intermediate arylhydroxylamine could be secured with the above mentioned substrates. However, with the carcinogen 4-nitroquinoline N-oxide, the hydroxylamine was found in high yield when incubated with rat liver post-mitochondrial fraction. Study of the kinetics of the reactions accounts for the failure to detect hydroxylamino intermediates with the aryl derivatives studied. With synthetic arylhydroxylamines as substrates, it was found that they were very rapidly reduced to the corresponding amines whereas 4-hydroxylaminoquinoline N-oxide was reduced only slowly. It seems, therefore, that the rate-limiting step in the reduction of nitroaryl derivatives to the amines is the first reduction of the nitro group to the hydroxylamino step. Subsequent reactions are fast, and thus it will be difficult to trap the intermediate product. This approach of assessing formation of hydroxylamine derivatives of aromatic compounds from the corresponding nitro derivatives cannot be used directly to predict carcinogenic hazards from nitroaryl compounds. We are now testing the potential carcinogenicity of 1-nitronaphthalene and related nitroaryl compounds.

Significance to Biomedical Research and the Program of the Institute:

By the utilization of certain chemical carcinogens, the molecular mechanism of steps leading to cancer is being explored. If the entire process is viewed as a series of steps going from 1) the primary interaction between an agent and a crucial cellular constituent or constituents, a reaction for which there are repair processes antagonizing the attack, followed by 2) multiplication of abnormal cells which in turn can 3) undergo further transformations, it is easy to visualize a number of points which with future effort could conceivably prevent or even reverse such interactions. Thus, the ultimate aim of these studies is to fully comprehend the sequence of complex reactions leading to cancer and hopefully to be in a position to prevent them under realistic conditions in man.

Proposed Course of Project:

The program of elucidating the biochemistry of carcinogenesis, our eventual goal, is a continuing, long-term effort. Certain promising leads which have been discussed in this account will be followed up in further experiments.

Honors and Awards

Dr. L. A. Poirier:

Member, Bioassay Segment

Chairman, Subgroup on Short-term Bioassay Systems

Consultant, Pharmaceuticals Manufacturing Association, on short-term bioassay systems

Publications

Lepage, R., Poirier, M. C., and Poirier, L. A.: One-carbon enzymes in the livers of rats during the chronic administration of 2-acetylaminofluorene, 4-dimethylaminoazobenzene and diethylnitrosamine. Cancer Res. (in press).

Matsushima, T., Grantham, P. H., Weisburger, E. K., and Weisburger, J. H.: Phenobarbital-mediated increase in ring- and N-hydroxylation of the carcinogen N-2-fluorenylacetamide, and decrease in amounts bound to liver DNA. Biochem. Pharmac. (in press).

Poirier, M. C., Lepage, R., Poirier, L. A., and Morris, H. P.: The enzymology in hepatomas and in the Walker Tumor 256 of the formation and inter-conversion of labile one-carbon groups. Cancer Res. (in press).

Weisburger, J. H.: Cancer and environment - Mechanisms of carcinogenesis with emphasis on aromatic amines. Proceedings of Symposium on Metabolism and Disease, Ottawa, Canada (in press).

Weisburger, J. H. and Weisburger, E. K.: N-Oxidation enzymes. In Brodie, B. B. and Gillette, J. R. (Eds.): Handbook of Experimental Pharmacology (Vol. 28), Part II. Concepts in Biochemical Pharmacology, New York/Heidelberg, Springer-Verlag, 1971, pp. 312-333.

Weisburger, J. H. and Weisburger, E. K.: Biochemical formation and pharmacological, toxicological and pathological properties of hydroxylamines and hydroxamic acids. Pharmacological Reviews (in press).

Weisburger, J. H., Yamamoto, R. S., Williams, G. M., Grantham, P. H., Matsushima, T., and Weisburger, E. K.: On the sulfate ester of N-hydroxy-N-2-fluorenylacetamide as a key ultimate hepatocarcinogen in the rat. Cancer Res. 32: 491-500, 1972.

Serial No. NCI-4620
1. Experimental Pathology Branch, OASDC, Etiology
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mode of Action of Chemical Carcinogens -- Chemical Investigations

Previous Serial Number: Same

Principal Investigator: E. K. Weisburger, Ph.D.

Other Investigators: J. H. Weisburger, Ph.D.

Cooperating Units: Laboratory of Chemistry, NHLI
Viral Carcinogenesis Branch, NCI
Drug Development Branch, NCI

Man Years:
Total: 0.7
Professional: 0.5
Other: 0.2

Project Description

Objectives:

In order to gain an understanding of the etiology of cancer, chemical studies are performed on the properties of certain carcinogens. Model studies on the reactions of chemical carcinogens with potential targets are performed.

Methods Employed:

Properties of chemicals acquired or synthesized for the bioassay of chemical carcinogens are investigated to determine the purity of the materials. In model experiments the reaction of certain of these agents, particularly their primary and ultimate reactive forms with select potential targets are studied. The materials produced are made available for related studies on the biochemistry and biology of the carcinogenic process. Methods for purification and analysis of various chemicals are applied.

Major Findings:

During the past year various compounds needed as reference materials in the study of acetanilide metabolism were synthesized, with emphasis on thiols, cysteines, or mercapturic acids. Samples of various carcinogens are being supplied for Dr. Huebner and associates for joint studies on viral-chemical interactions in carcinogenesis.

Standard carcinogens were furnished to both NCI and other investigators.

With the cooperation of the Drug Development Branch, representative commercially available carcinogens are being checked for purity as part of a project to furnish purified samples of standard carcinogens to various investigators.

Significance to Biomedical Research and the Program of the Institute:

The studies performed are a necessary adjunct to the broad programs on the mechanism of action of chemical carcinogens pursued in this Section.

Proposed Course of Project:

This project will continue along the same lines as previously.

Honors and Awards

E. K. Weisburger:

Assistant editor-in-chief, JOURNAL OF THE NATIONAL CANCER INSTITUTE, 1971--

Editorial Board, EXCERPTA MEDICA (Cancer)

Board of Trustees, Lebanon Valley College

Staff Consultant, CARCINOGENESIS ABSTRACTS

Listed in "Two Thousand Women of Achievement, 1971"

Guest lecturer, USDA Graduate School, February 28, 1971

Member, Information and Resources Segment

Invited participant, "Arene Oxide" Conference, sponsored by Roche Institute of Molecular Biology

Serial No. NCI-4680
1. Experimental Pathology Branch, OASDC, Etiology
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mode of Action of Chemical Carcinogens -- Development and Application of In Vitro Systems Involving Epithelial Cells

Previous Serial Number: Same

Principal Investigators: R. Kroes, D.V.M., Ph.D. and J. H. Weisburger, Ph.D.

Other Investigators: G. M. Williams, M. D., J. Elliott, and J. Idoine, M.S.

Cooperating Units: Dr. K. Stromberg (Laboratory of Biology, NCI)
Dr. R. J. Huebner (Viral Carcinogenesis Branch, NCI)
Dr. Freeman and Dr. Rhim (Microbiological Associates)

Man Years:

Total:	1.8
Professional:	0.6
Other:	1.2

Project Description

Objectives:

Neoplasia in man and animals involves mostly epithelial cells. Thus far efforts to grow epithelial cells in vitro under conditions required for studies of neoplastic transformation have been mostly unsuccessful. Many efforts along these lines have been at the level of cultures derived from whole embryos or of fibroblasts. The transformation obtained in such system with a few chemicals resulted in the production of mesenchymal tumors. Our principal aim was to secure cultures of epithelial cells from liver for studies of their biochemical properties and attempts at transformation, which would yield carcinoma, the principal type of neoplasia in man and animals. Once established such systems could serve to not only gain insight into the mechanism of action of chemical carcinogens under readily controlled conditions, but hopefully also assist in developing quick and reliable bioassay procedures for chemical carcinogens.

Methods Employed:

Classical tissue culture methods were applied but modified in such a manner to select epithelial cells and maintain them under conditions where continuing growth and proliferation was maintained. Procedures were applied to characterize these cells by biochemical and morphological tests. Select carcinogens were added and the results evaluated with reference to specific criteria.

The activity of certain key enzymes, particularly those involved in the metabolism of chemical carcinogens located on the endoplasmic reticulum is determined on cell systems treated in vitro with chemical carcinogens with and without infection by viral agents and also after treating animals with chemicals.

Major Findings:

1. Transformation by various chemical carcinogens of epithelial hepatic cell cultures and development of hepatocellular carcinoma after injection of transformed cells into syngeneic hosts. The successful development of reliable means of growing epithelial liver cells from rats in vitro have been detailed previously.

Treatment of liver cell systems with aflatoxin B_1, dimethylnitrosamine, N-nitrosomethylurea, N-hydroxy-N-2-fluorenylacetamide or 7,12-dimethylbenzanthracene for extended periods of time have in some instances led to alterations in morphology, in the growth rate, and in a few cases of the karyotype of the epithelial cell systems. However, in contrast to embryonic cell cultures of fibroblasts treated with carcinogens in vitro, the changes seen in our epithelial cell cultures proved unreliable indicators of any alteration towards a malignant state. Routine periodic injection of from 5 to 20 million cells into syngeneic hosts, either newborn or x-radiated weanlings, led to the development of transplantable tumors with cell populations treated with the carcinogens mentioned above. Comparable injection of cultures of untreated cells failed to yield any tumors.

The tumors in almost all cases were diagnosed as hepatomas or hepatocellular carcinomas. A few were mixed cell tumors or fibrosarcomas. Except for a similar study carried forward in Japan with the carcinogen 4-nitroquinoline oxide, our findings constitute unique results involving the production of carcinoma like that seen in man after transformation of cell cultures with a variety of carcinogens. Refinement of the technique with respect to shortening the exposure time in vitro, and the assessment of the time of transformation by appropriate techniques are now areas of primary consideration.

Significant results with respect to the biochemical properties and ultrastructural aspects of these epithelial cell systems were also obtained. Histochemical determination of certain key enzymes suggested that there was a difference in enzyme activity in actively growing cells compared to cells with reduced growth rate due to contact inhibition in monolayers. A further examination by histochemical and biochemical techniques has fully confirmed this finding. For example, benzpyrene hydroxylase is higher in cells present in monolayers compared to rapidly multiplying cells. Inasmuch as many chemicals tested require enzymic activation, exposures of rapidly growing cells compared to cells which have not been passaged are now being studied.

Hepatic cells may not require exogenous glucose for maintenance of growth. From the fact that some of our cell populations can develop in low glucose, and indeed also for some period of time in glucose-free media would suggest that these cells do contain the necessary enzymic apparatus to progress without exogenous glucose. More information in this system is however required as to the underlying mechanism.

2. Comparison of enzyme activity in virus-infected cell systems and control cultures. Huebner and associates have devised means of examining in vitro transformation in cell systems. Under certain conditions transformation by their criteria was obtained only when cells were treated simultaneously with a chemical carcinogen and a viral preparation, most often the Rauscher C type murine-leukemia virus. It seemed important to ask the question whether cells infected with virus had a different enzymic potential to metabolize, and indeed to activate chemical carcinogens than cells not so infected. In cooperation with Dr. Huebner and associates, particularly Dr. Freeman and Dr. Rhim at Microbiological Associates, rat embryo cell cultures were infected with the Rauscher leukemia virus. With the cooperation of staff of Dr. Gelboin, Chemistry Branch, benzo(a)pyrene hydroxylase, a typical carcinogen metabolizing enzyme, was determined in virus-infected cell systems, and appropriate controls. In many but not all of the tests conducted at various times post-infection with virus, there were appreciable and indeed sometimes highly significant increases in benzpyrene hydroxylase activity in infected cells. These preliminary observations need to be confirmed, and extended with this and other carcinogens as substrate. If virus infection does alter the drug metabolizing enzyme system, the finding of synergism between viruses and chemical would receive an additional explanation, based on the biochemical potential of the cell.

Our own lines of normal epithelial hepatic cells are currently being infected with Rauscher virus to conduct similar studies in a specific clean epithelial cell system.

Significance to Biomedical Research and the Program of the Institute:

An assay system which can be designed to give results more rapidly, more specifically, and more reliably than the customary long-term tests for carcinogenicity is a highly desirable aim in the field of chemical

carcinogenesis with the dual purpose of studying fundamental phenomena and also the detection of harmful agents in our environment. Full exploitation of workable and realistic in vitro systems in this respect has not received the attention it deserves. It is our goal to develop such systems with the aim of furthering efforts leading to the prevention of cancer. An additional advantage of such in vitro systems is that eventually they can be established utilizing cells from man. Hence carcinogenesis in vitro with human cells avoids the question of species difference, so often raised in evaluating the significance of carcinogen bioassay systems.

Proposed Course of Project:

The important in vitro test systems involving epithelial cells will be carefully examined for suitability in carcinogen screening systems. Also the relevant morphological and biochemical properties in normal and carcinogen treated cells will be subject to thorough scrutiny. Further extensive efforts will deal with virus-chemical interactions in cell systems, both as a more sensitive bioassay test, and also to explore basic biochemical mechanisms of such interactions.

Honors and Awards

Dr. G. M. Williams, IARC Fellow, The Wenner-Gren Institute, University of Stockholm, Sweden

Dr. R. Kroes, IARC Fellow, Experimental Pathology Branch, Carcinogen Screening Section, National Cancer Institute

Publications

Williams, G. M., Weisburger, E. K., and Weisburger, J. H.: Isolation and long-term cell cultures of epithelial-like cells from rat liver. Exptl. Cell Res. 69: 106-112, 1971.

Serial No. NCI-4688
1. Experimental Pathology Branch, OASDC, Etiology
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mode of Action of Chemical Carcinogens -- Investigations of Systems Leading to Cancer in Colon and Other Digestive Organs

Previous Serial Number: Same

Principal Investigator: J. H. Weisburger, Ph.D.

Other Investigators: R. S. Yamamoto, Sc.D., C. Lingemann, M. D., C. Brown, M.S.

Cooperating Units: Dr. J. W. Berg (Epidemiologic Pathology Unit, Carcinogenesis, NCI)

Man Years:

Total:	2.1
Professional:	0.6
Other:	1.5

Project Description

Objectives:

In the United States the mortality rate due to colon and rectum cancer in males is second only to lung cancer, and in females second to breast cancer. In incidence, colon cancer is third after skin and lung in men, and in women, after breast and uterus. In some other countries like in Western Europe, the relationship is similar. However, in other countries such as Japan and Africa, colon cancer is much lower, thus suggesting that environmental factors are connected with the etiology of this important disease. Cancer of the pancreas is increasing and nothing is known precisely on causative factors. Because of the absence of adequate tools to induce colon and rectal cancer in animals, model studies could not be performed. However, recently several new agents were discovered by which colon and in part rectum cancer can be reproducibly induced in animal systems. There is a possibility that cancer of the pancreas may also result. The goal of the studies to be described is to evaluate how select environmental factors affect the production of cancer of the colon, rectum and perhaps pancreas in animal systems, and thereby gain insight into conditions possibly operating in man.

Methods Employed:

Rats, mice and also other rodent species were treated appropriately with chemicals which have been shown to lead to colon cancer. They include 1,2-dimethylhydrazine, azoxymethane, methylnitrosourea, and 2',3-dimethyl-4-aminobiphenyl. The animals are placed on diets mimicking those utilized in various parts of the world as regards quantity and quality of protein, fat, carbohydrate, micronutrient and trace element. Other variables examined initially are route of intake of the agent as well as dose levels. After appropriate periods of time animals were carefully autopsied and the lesions produced diagnosed microscopically.

Major Findings:

1. Effect of diet on tumor formation in the intestinal tract. Twice weekly injection of dimethylhydrazine at a 0.2 mM/kg level followed by one injection per week for 10 weeks and an additional period on control diet for 6 to 8 more weeks yielded tumors in the ear duct, small intestine and in the large intestine in varying proportions. Animals on the high fat or high fat-high protein diet were more susceptible to the toxic effect of the agent, and there were relatively few survivors. However, even in these groups there were sizeable numbers of animals with ear duct tumors, and all the rats surviving for more than 25 weeks had colon cancer. Animals fed lower levels of fat and the basal or high level of protein exhibited tumors at the 3 main sites, with relatively little difference as a function of diet. Rats given the basal diet and added tryptophan as well as those on the high protein diet appeared to present additional lesions in the pancreas which remain to be analyzed further.

In addition to tumor formation, the treated rats had intraperitoneal bleeding, resulting often in low hematocrits. There was testicular atrophy, kidney damage, and some evidence of liver damage.

Female rats were more resistant to the toxic effects and more animals survived on all of the diets. There appeared to be more of the pancreatic lesions in the females compared to males.

Additional studies involving shorter treatment at lower dose levels indicate in a preliminary way that the incidence of ear duct tumors is unaffected, that colon cancers result in similar yields, but that the small intestinal tumors, seen with high dose levels, were somewhat reduced.

Similar results were obtained with the colon carcinogen, azoxymethane. Here also, high levels yielded tumors in the ear duct, small intestine and the colon, and indeed with this agent under our conditions there seem to be more duodenal tumors as compared to tests where the carcinogen was 1,2-dimethyl-hydrazine.

All of these tests are being performed in the highly inbred Fischer F344 rat. Certain of the colon and duodenal tumors were transplanted in syngeneic systems under a variety of conditions. So far no transplantable intestinal tumor has resulted. However, at this time a few of the s.c. implants are still present and are being watched. There is no experimental transplantable intestinal cancer available anywhere.

2. Toxicity studies on 1,2-dimethylhydrazine and azoxymethane in various strains of mice. Several dose levels of the two agents were injected into 7 inbred and random-bred strains of mice. Preliminary observations indicate that the tolerated dose is similar, irrespective of mouse strain.

3. Intestinal and fecal enzymes as a function of diet. Geographic differences in colon cancer incidence have been related to diet which in turn appear to be a function of transit time of foodstuffs as well as of the bulk of diets. Comparisons have been made of individuals on different diets with respect to the composition of the bacterial flora in the intestinal tract. We asked the question whether diet might lead to different enzyme potential of intestinal flora qualitatively or quantitatively.

In a preliminary way two enzymes which we were equipped to study, β-glucuronidase and azo dye reductase, were estimated in the cecal contents and the stools of rats on a high bulk commercial chow, and a low residue semi-synthetic diet, the A diet of Dr. R. Yamamoto. β-Glucuronidase had been found previously (Biochem. Pharmac. 19: 173, 1970) to be low in germ-free rats and had a pH optimum of approximately 5, typical of the mammalian enzyme. In conventional animals, the enzyme had much higher levels and a pH optimum between 6 and 7, typical of the bacterial enzyme. Most chemical carcinogens are metabolized in the liver, in great part to conjugates with glucuronic acid, and as such reach the intestinal tract via the bile. Thus, β-glucuronidase is a key enzyme for the liberation of active carcinogens in the gut.

We now find that both enzymes studied, β-glucuronidase and azo dye reductase, had quite a variable specific activity, in units per mg of intestinal contents or of feces. However, there were sizeable differences in total amounts of activity, being much higher in the animals fed chow compared to the animals on the semi-synthetic diet. This is accounted for by the much larger bulk of the contents and of the stools in the animals on chow. Additional studies in this area, especially in respect to enzyme metabolizing steroids in bile are of interest.

Significance to Biomedical Research and the Program of the Institute:

In view of the importance of colon cancer in man, model studies on the mechanisms of colon cancer formation are of paramount importance, especially since epidemiologic and migrant data do indicate that colon cancer almost certainly is due to environmental factors. The systems now studied and related efforts to be developed extensively in the peripheral areas such as the enzymology of the colon and the corresponding tumors, immunological factors, including the occurrence of carcino-embryonic antigens, the

development of in vitro systems related to colon carcinogenesis and the like should in due time produce an accurate picture of factors controlling colon cancer induction and development so that this important disease in man would eventually yield to realistic preventive efforts.

Proposed Course of Project:

Numerous relevant variables need to be studied in the induction of colon cancer in our model systems. Several other carcinogens must be applied, including some endogenously formed, possibly from a combination of nitrite and an appropriate secondary amine derivative, in order to permit eventual extrapolation to man. Systems must be studied in other species as well. Inasmuch as this is a relatively uncharted road, many different points of attack are possible. On the basis of our past experience with other organ systems such as liver, some selectivity is possible. Also guidance will be obtained from a study of factors favoring the presence of colon cancer in man as a function of the environment, so that model studies will be undertaken which will yield significant information with hopefully a minimum of waste motion. Inasmuch as the bacterial flora in the gut is almost certainly involved in the development of colon cancer, this element deserves exploration. Rather than attacking the problem indirectly by examining the complex bacterial flora as such, we hope to devise methods to assess the relevant enzyme capability of a given bacterial flora, since the enzymic potential is actually the key element. Further to be considered are germ-free or antibiotic-treated systems, as well as animals deliberately fed diets to provide high or low bulk in the intestinal tract. Similar test series will be directed toward the study of cancer of the pancreas, and indeed other digestive organs, as model systems can be generated.

Publications

Weisburger, J. H.: Colon carcinogens: Their metabolism and mode of action. Cancer 28: 60-70, 1971.

Weisburger, J. H.: Model studies on the etiology of colon cancer. Gann Monograph (in press).

Serial No. NCI-4500
1. Experimental Pathology Branch, OASDC, Etiology
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mode of Action of Chemical Carcinogens -- The Mechanism of the Appearance of α-Fetoproteins (AFP) in Serum of Rats Given Chemical Carcinogens. Immunosuppression and Chemical Carcinogenesis.

Previous Serial Number: None

Principal Investigator: R. Kroes, D.V.M., Ph.D.

Other Investigators: G. M. Williams, M. D. and J. H. Weisburger, Ph.D.

Cooperating Units: None

Man Years:
Total: 1.2
Professional: 0.7
Other: 0.5

Project Description

Objectives:

A fetal globulin, α-fetoprotein (AFP) has been demonstrated to be present in the serum of some patients with hepatocellular carcinoma, and a few other lesions such as teratomas. Depending on geographic location, the percentage of AFP-positive patients varies from 30% in some areas like the United States or Great Britain, to 80% in Africa. The presence of AFP is being developed as a diagnostic tool for hepatocellular carcinomas. A number of questions on the mechanism of formation, of the derepression of this fetal protein are of interest in relation to the etiology of hepatocellular carcinoma in man. Do all hepatocarcinogens lead to tumors elaborating AFP? Does age play a role? Does the morphology and differentiation of the tumor affect production? Some of these basic questions were investigated in rats.

The effect of specific immunosuppression by antilymphocytic globulin on liver and colon carcinogenesis in rats was studied.

Methods Employed:

Utilizing amniotic fluid of inbred female Fischer strain rats at 16 to 20 days of gestation as antigen, antiserum was raised in rabbits. The antiserum was purified by select and specific techniques to secure in a series of steps an AFP-specific antiserum. Double diffusion (Ouchterlony) techniques were utilized to assess the presence of AFP in serum of treated and control rats. In some cases confirmatory experiments were performed by electrophoresis and immunoelectrophoresis on cellulose acetate strips or on agarose coated slides.

Groups of rats injected with ALG were fed a hepato- and a colon carcinogen to evaluate the effect of such immunosuppressive treatment on cancer development.

Major Findings:

1. Presence of AFP in serum of rats fed various hepatocarcinogens or hepatotoxins. By all criteria examined the antiserum obtained by our technique was specific for AFP and failed to respond to other antigens.

The completely novel discovery was made that within 3 to 4 weeks of initiation of feeding the one hepatocarcinogen, 3'-methyl-4-dimethylaminoazobenzene (3'-MDAB), AFP-positive sera were noted. If the carcinogen was fed for only 5 weeks and the rats returned to control diet, the titer of the antigen dropped within the next 2 weeks and failed to reappear over a 20 to 30 week test period. However, if the carcinogen was fed for 10 weeks a few of the rats on test continued with positive sera and these, at autopsy at 20 weeks, exhibited large primary hepatocellular carcinomas. The remainder of the rats fed 3'-MDAB for 10 weeks also showed disappearance of the antigen over a two-week period, but several weeks later AFP reappeared. At autopsy all rats with positive sera also had hepatoma. There were, however, a few rats with hepatoma which had negative sera. When the carcinogen was fed throughout the experimental period some of the rats presented declining titers between the 13th and 17th week of the test and then became more positive. All rats with positive titers had hepatoma. In this instance also, a few rats with hepatoma had no detectable antigen. Liver damage as such failed to yield detectable AFP levels, for rats treated with the hepatotoxin α-naphthylisocyanate never had AFP although they had all the characteristics, jaundice and liver damage expected.

In a dose-response study, animals fed very high levels, 1200 ppm 3'-MDAB had positive titers after the first week. On the other hand, rats fed only 150 ppm showed no detectable antigen. The time of appearance and the period during which positive titers were seen was related to the dose level of the carcinogen.

A related carcinogen, 2'-MDAB also yielded positive titers but the non-carcinogenic analog 2-MDAB did not, nor did animals given carbon tetrachloride.

Rats given diethylnitrosamine had positive AFP tests. High levels (4 ppm) but not lower levels (2 and 0.2 ppm) of the important hepatocarcinogen aflatoxin B_1, the carcinogen N-2-fluorenylacetamide, but not the active metabolite N-hydroxy-N-2-fluorenylacetamide, gave rise to the precocious production of AFP. There were select and distinct differences in the time of the early production of AFP and the period during which positive titers were seen while animals were continuously treated with these agents.

In addition to the prompt derepression of AFP production by various carcinogens discussed above, studies are currently underway with all of these agents to establish whether there is a difference in AFP production in animals bearing tumors induced by these different agents. A preliminary observation with sera obtained from the laboratories of Drs. Wogan and Newberne at MIT through the courtesy of Dr. T. Waldmann, NCI, from animals bearing tumors induced with aflatoxin B_1, it would appear that such tumors yield positive sera, in contrast to several negative studies in the literature. On the other hand, it would appear thus far that rats bearing tumors induced by N-hydroxy-N-2-fluorenylacetamide show low titers or only a small percentage of positive sera.

From human data, the question whether age of the patient plays a role in determining positive titers of AFP has been controversial. In our model system, utilizing 3'-MDAB as carcinogen, rats aged 6 weeks, 9 weeks, 12 weeks, 9 months and 15 months exhibited a similar response as regards the precocious appearance of positive titers of AFP. There was a slight delay in the appearance in the older animals, being 4 to 5 weeks after initiation of carcinogen, rather than 3 to 4 weeks.

One test dealt with the possible mechanism of the precocious production of AFP. The concept was investigated that the precocious appearance of AFP was actually due to the presence of early tumor cells, and the disappearance of AFP might be related to the suppression of the tumor cells by the immuno-competent host. We investigated this idea by giving carcinogen for 5 weeks during which time AFP was uniformly positive, then stopping carcinogen. To one-half of the animals we injected the gamma fraction of antilymphocytic serum to suppress the immunological response. However, AFP disappeared in this group in almost the same period (1 to 2 weeks) as in appropriate controls.

In all our studies, comparisons were made between the histologic aspect of liver at various periods of time during which carcinogen induced or failed to induce AFP. These studies are not yet concluded. It could be that the "oval cells" may relate to positive sera. It is fairly certain already, however, that AFP production in animals bearing liver tumors is not necessarily related to the morphology and state of differentiation of the tumor. Positive sera were seen with highly differentiated tumors as well as with anaplastic carcinomas.

2. Effect of immunosuppression on the induction of liver and colon tumors by chemical carcinogens. There is considerable controversy whether the immunocompetence of the host modifies and controls tumor development. Some tests indicated that tumor induction was facilitated in immunosuppressed animal systems whereas others found no effect. Most of the previous studies have dealt with sarcoma formation after s.c. injection of polycyclic hydrocarbons or skin tumor formation in mice with these same agents.

New tests were initiated in rats given either N-hydroxy-N-2-fluorenylacetamide which would yield liver tumors or azoxymethane which cause also colon cancer. Some of the animals received immunosuppressive treatment with the gamma fraction of antilymphocytic serum. There were appropriate controls. Definitive results from these tests will be available in the late spring.

Significance to Biomedical Research and the Program of the Institute:

The current test series were inspired by differences in the presence of a diagnostic antigen, α-fetoprotein (AFP) in the serum of human patients with liver cancer. The etiology of liver cancer in different parts of the world is unknown but presumably rests on a variety of inciting factors and chemical carcinogens. Model studies to pinpoint specific carcinogenic agents which would give rise to AFP and others which would not might give clues to etiologic factors operating in man in various parts of the world. At the same time an understanding of the factors controlling the release of a fetal antigen from normal or cancer cells might further our understanding of the mechanisms of carcinogenesis and thus eventually yield information which can be used to control and indeed prevent cancer development. Studies on the effect of immunocompetence in the carcinogenic process likewise bear on these same problems and their resolution would certainly augment means of controlling the progress of the disease.

Proposed Course of Project:

More information is needed on which cells are involved in precocious release and production of AFP, as well as on the later development of AFP producing cells in the tumors induced by various chemical carcinogens. Inhibitors and inducers of protein synthesis will be applied to a study of the precocious release in order to formulate theses on the underlying mechanisms. Further evidence will be needed on the key questions of immunocompetence in relation to the carcinogenic process.

Publications

Kroes, R., Williams, G. M., and Weisburger, J. H.: Early appearance of serum α-fetoprotein during hepatocarcinogenesis, age of rats, and extent of treatment with 3'-methyl-4-dimethylaminoazobenzene. Cancer Res. (in press).

III. *COLLABORATIVE PROGRAM REPORTS*

SUMMARY REPORT

OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR FOR CARCINOGENESIS

Fiscal Year 1972

Two large interdisciplinary contracts are reported under this Office: The contract with the AEC (Oak Ridge National Laboratory) and the contract with the University of Nebraska (Eppley Institute for Research in Cancer). Both of these contracts include several projects that relate to the program of individual segments. The budget has been apportioned to the respective segments which provide the scientific coordination with their respective research activities. However, in order to present a cohesive summary of the progress accomplished under these large institutional contracts, their narrative reports are included here.

CONTRACT NARRATIVES

AEC-NCI INTERAGENCY AGREEMENT, OAK RIDGE NATIONAL LABORATORY (NCI-FS-64-13)

Title: NCI-AEC Carcinogenesis Program - Research on Co-Carcinogenesis

Contractor's Project Director: Dr. James Liverman

Project Officer (NCI): Dr. Allen Heim

Objectives: The broad objectives are as follows: (A) to carry out large scale co-carcinogenesis studies in exposure chambers with controlled atmospheres containing selected chemical agents to be tested for direct carcinogenic effects on respiratory tissues or for co-carcinogenic effects with known carcinogens, radiation, and viruses; (B) to bring to bear on problems of chemical and viral carcinogenesis the diverse scientific talents of the staff of the ORNL, Biology Division, and the scientific and engineering expertise of other ORNL components. A major part of the funding is associated with the first objective.

Specific objectives are as follows: (1) to develop and define animal models for study of the pathogenesis of human lung cancer with emphasis on interaction of exogenous and endogenous factors, using inhalation exposures as well as other methods for respiratory carcinogenesis studies; (2) to obtain comparative data on mutagenic and carcinogenic responses to various chemicals in microbial systems and in mammalian cells; (3) to develop tools to permit detailed study of the role of the genes of mammalian somatic cells in transformation to malignancy by chemical or viral agents; (4) to analyze the mechanisms for repair of damage to DNA by chemicals or irradiation in mammalian cells and the role of such damage and repair in carcinogenesis; (5) to analyze the interaction of host immune mechanisms with chemical carcinogens and RNA tumor viruses; (6) to detect and characterize changes in DNA, RNA and protein synthesis in cells and tissues transformed by chemicals

or by oncogenic viruses; (7) to analyze the mechanisms by which gene expression is regulated in mammalian cells, with special attention to regulation of expression of oncogenic viruses; (8) to characterize cellular changes during carcinogenesis by the Shope virus and its mutants and to develop the use of passenger viruses to transmit specific genetic information; (9) to investigate the relationship between embryogenesis and oncogenesis, especially as regards the role of C type viruses in both processes.

As stated above, one of the major objectives is to incorporate the scientific talents of the ORNL staff into the Carcinogenesis Program. The degree to which this has been accomplished may best be realized by presenting the objectives, major findings and significance of each of the particular efforts separately, keeping in mind that the strength of the program at ORNL lies in the fact that these efforts are coordinated.

(1) *Respiratory Carcinogenesis*

Specific Project Director: Dr. Paul Nettesheim

Objectives: To analyze co-carcinogenic effects of physical and chemical agents in the induction of lung cancer. Identification and study of those environmental agents (e.g., irritant gases and dusts, infectious microbes) which may increase the susceptibility of respiratory tissues to normally ineffective doses of carcinogen or which may alter the "effective" tumorigenic dose by affecting distribution, deposition, and/or clearance of inhaled carcinogenic particulates.

Major Findings: Inhalation of ferric oxide dust appears to enhance the peripheral lung tumor response elicited by DEN. The lung tumor incidence in rats with spontaneous murine pneumonia and given N-nitrosoheptamethyleneimine was approximately twice that in SPF rats given the same amount of carcinogen. Germ-free rats developed essentially the same types of tumors as the SPF and infected animals, indicating that the presence of microbial flora is not essential to nitrosamine metabolism. A method for studying pulmonary cytology in rats and hamsters by tracheal washing has been developed and is being used with encouraging results. It is appropriate for studies of lung cancer morphogenesis and can be used to correlate the experimental histomorphogenesis of non-malignant and malignant lesions with the exfoliative cytology of the lung. Rats injected intratracheally with BaP or MCA, with or without ferric oxide, respond differently than hamsters injected intratracheally with the same material: the amount of carcinogen required for tumor induction is much less in rats, the site of metaplastic response is different, and the time required for a response is shorter. Mice with influenza virus pneumonia have a marked and persistent defect in their alveolar clearance of $^{51}Cr_2O_3$ particles.

Significance to Biomedical Research and the Program of the Institute: Epidemiologic data indicate a multifactorial etiology in human lung cancer. This work is a study, using animal models, of the role of inhaled chemicals and of physical and biological agents influencing the respiratory tract in the development of lung cancers. It is expected that this approach will help to define the environmental agents acting to induce lung cancer in humans.

Current Annual Level: $710,300

(2) *Mutagenicity of Carcinogens*

Specific Project Director: Dr. Frederick de Serres

Objectives: To determine (1) whether there is a correlation between carcinogenicity and mutagenicity and (2) whether there is a correlation between carcinogenicity and the production of a particular type of genetic alteration.

Major Findings: Of 14 carcinogens tested for mutagenic activity in conidial suspensions or vegetative cultures of *Neurospora crassa*, 13 proved to be mutagenic. *In vitro* systems have been developed that give the same type of carcinogen activation normally found *in vivo* in the mammalian liver or in other organs. With these systems and the host-mediated assay, remote as well as proximal carcinogens, have been found to be mutagenic.

Significance to Biomedical Research and the Program of the Institute: This work has provided more reliable assays for detection of mutagenic activity of carcinogens. Analyses completed to date show a strong correlation of mutagenic activity with carcinogenicity. This provides support for the concept of somatic cell mutation as an essential component in carcinogenesis, and may lead to a simplified screening method for carcinogenicity of suspect chemicals.

Current Annual Level: $121,400

(3) *Somatic Cell Genetics*

Specific Project Director: Dr. E. H. Y. Chu

Objectives: (1) To develop techniques for induction, selection, and analysis of gene mutations in human and other mammalian cell cultures; (2) to estimate the quantitative cytogenetic effects of radiation and chemicals in mammalian cell systems; (3) to find possible relationships between mutagenesis and carcinogenesis.

Major Findings: Auxotrophic mutants requiring purine, pyrimidine, or specific amino acids or sugars have been isolated from cultured Chinese hamster cells and characterized. One group of mutants is incapable of utilizing galactose for growth because of enzyme deficiencies in galactose metabolism. Inter-allelic complementation between mutants resistant to cytosine arabinoside has been demonstrated. N-acetoxy-2-acetylaminofluorene, an ultimate carcinogen derived from 2-acetylaminofluorene, has been found to be highly mutagenic (as have other carcinogenic derivatives of parent compounds). Reverse-mutation experiments indicate that mutants induced by this compound are mostly deletions or base-pair transitions.

Significance to Biomedical Research and the Program of the Institute: Development of somatic cell genetics with the objective of defining the role of specific genes in carcinogenesis is an essential component of the Institute's research program.

Current Annual Level: $76,400

(4) *Repair Mechanisms in Carcinogenesis*

Specific Project Director: Dr. R. B. Setlow

Objectives: (1) To understand the role of DNA-repair mechanisms in determining the response of cells to carcinogenic agents; (2) to elucidate the role of specific UV-induced changes in DNA (pyrimidine dimers) in UV-induced cancer.

Major Findings: Using a newly developed bromouracil photolysis technique for measuring DNA repair, the following has been demonstrated: (A) There is extensive repair in the DNA of normal human fibroblasts after treatment with N-acetoxy-AAF; in fibroblasts and tumor cells of individuals with xeroderma pigmentosum the repair is an order of magnitude less, indicating that the enzyme defect in these cells is not confined to UV damage but extends to chemical alterations in DNA. (B) ICR-170-induced damage to DNA is repaired in normal cells but not in xeroderma cells. (C) 4-Nitroquinoline oxide results in a complex spectrum of damage, one part of which is repaired in both normal and xeroderma cells, the other only in normal cells.

Significance to Biomedical Research and the Program of the Institute: Chemically-induced cancers may develop as a result of failure of cellular mechanisms for repair of damage to DNA. This work provides knowledge of the cellular mechanisms operative in repair and may lead to measures for prevention of cancers induced by chemical or physical agents.

Current Annual Level: $115,000

(5) *Immunology of Carcinogens*

Specific Project Director: Dr. Michael Hanna, Jr.

Objectives: To correlate both the histopathologic aspects of the systemic immune response and anatomic factors involving the progressive growth of chemically-induced primary and secondary tumors with functional immunologic factors directed against successful tumor growth.

Major Findings: Interdermal tumors transplanted to guinea pigs completely regressed after being treated with BCG, and regional lymph node metastasis was completely eliminated. The mechanism of regression appears to be a BCG-mediated granulomatous reaction at both the tumor site and the regional lymph node, with histiocytes which are apparently the major effector cells in this reaction. Treatment of the transplanted tumors with vaccinia virus, oxazolone, or turpentine failed to produce regression of tumor growth or elimination of metastasis.

Significance to Biomedical Research and the Program of the Institute: This work provides an essential analysis at the histological and pathologic level of the role of the immune system in combating development and metastasis of chemically-induced tumors. It may lead to a more definitive mode of cancer therapy based upon fortification of the capacity to reject tumors by immunological mechanisms.

Current Annual Level: $120,000

Proposed Course: A new program aimed at establishing a good experimental model system for studying the use of inhalation methods in respiratory carcinogenesis is being initiated. It will examine the role of compounds such as nitrogen dioxide, acrolein, and formaldehyde as cofactors. Most of the studies are long time and will be continued essentially along the lines described above, each seeking to probe more into detail. The program in the "Mutagenicity of Carcinogens" will probably be curtailed because the principal staff members will be leaving ORNL.

Effective with Fiscal Year 1972, the responsibility for funding and monitoring this Program has been assumed jointly by Carcinogenesis and Viral Oncology. This report contains information related to those projects in the area of Carcinogenesis.

Date Contract Initiated: January 14, 1963

Current Annual Level: $1,143,100

UNIVERSITY OF NEBRASKA (EPPLEY INSTITUTE FOR RESEARCH IN CANCER) (NIH-NCI-E-68-959)

Title: A Resource for Carcinogenesis Bioassays and Related Research

Contractor's Project Director: Dr. Philippe Shubik

Project Officer (NCI): Dr. Gio Gori

Objectives: The purpose of this contract is twofold in nature: the bioassay of agents suspected of being carcinogenic and fundamental research on mechanisms of carcinogenesis.

(1) *Bioassay* - Compounds are selected for carcinogenic screening on the basis of several criteria, including (a) suspicion as a result of epidemiological studies uncovering suspected high risk to cancer in defined environmental exposures; (b) chemical properties and structures or physical properties suggesting a high index of suspicion for potential carcinogenic potency, and (c) potential or actual universal distribution or use of agents suggesting the need for carcinogenic bioassay. The techniques of bioassay include administration by various routes. Selection of various modes of administration is determined by properties of the materials, comparative response in various species, determination of dose response parameters, and the desire for maximum extrapolation to man. Protocols for these experiments take into account the current state of knowledge in the field as well as the properties of the agents being tested. Experimental design also utilizes the skills of the chemistry group and provides data analysis systems. A large number of compounds are under bioassay, using several dose levels and multiple species.

(2) *Research on the Mechanism of Carcinogenesis* - Objectives include fundamental research at the chemical and host levels for the purpose of

elucidating biological mechanisms involved in the carcinogenic process and to identify the factors controlling or modifying the natural history of induced neoplasms. Methods employed include the determinations of anatomic and metabolic fates of environmental carcinogens to determine sites of elective localization and paths of detoxification and excretion. Identification of the active form of the carcinogen, characterization of hormonal or genetic host factors critical to the carcinogenic responses and comparison of response in different species are integral parts of this program. Molecular aspects of carcinogenesis are actively investigated.

Major Findings: (1) *Bioassays* - The status of the bioassay efforts may be summarized as follows: (a) The discovery of the carcinogenicity of the widely used drug Metronidazole (Flagyl); (b) The demonstration that five substituted compounds of the hydrazine studies series are carcinogenic. Some of them constitute environmental hazards such as hydrazine, methylhydrazine and 1,1-dimethylhydrazine. All three are components of rocket fuels (the carcinogenicity of methylhydrazine was proven the first time); (c) Three groups of food additives or contaminants are under test, including artificial sweeteners in hamsters, aflatoxins in rats, and nitrite in combination with various amines in mice and rats. In mice piperazine, morpholine, and methylaniline in combination with sodium nitrite significantly enhanced the development of lung adenomas. The results with nitrites and amines in rats are expected in mid-1972. The tests with artificial sweeteners are at an early stage and the aflatoxin studies are finishing; (d) Lung bioassays of eight aromatic hydrocarbons (some of which are components of tobacco tar), six metallic dusts in combination with a carcinogen and seven carcinogenic compounds in various combinations are underway in hamsters; (e) The observation that dibutylnitrosamine is one of the most potent bladder carcinogens. The demonstration that the Syrian golden hamster is an ideal species for studies on the formation of tumors in bladder; and (f) The finding that 7-H-dibenz[*c,g*]carbazole, a component of cigarette tobacco tar, is a particularly potent lung carcinogen.

(2) *Research on the Mechanism of Carcinogenesis* - Selective examples of research projects include the following: (a) *Nitrosamine Studies:* (1) Induction of lung tumors in mice by secondary amines and nitrite which suggest *in vivo* nitrosation with the formation of carcinogenic nitrosamines. (2) The *in vitro* demonstration of nitrosation of various drugs (oxitetracyclin, etc.). (3) The observation that nitrosation of certain amines to form carcinogenic nitrosamines can be effectively prevented by concurrent administration of vitamin C. (4) The elucidation of the kinetics (physicochemical) of nitrosation of secondary amines. (b) *Hydrazine Studies:* The induction of high incidences of blood vessel neoplasms by the two dimethylhydrazines. This provides a model system for future investigations. The clarification of the ultrastructure and pathogenesis of the vascular tumor. (c) *Lung Carcinogenesis:* The morphological (light and electron microscopic) demonstration of the cell type and ultrastructure of tumors of tracheobronchial system. Clarification of the mechanism of tumor formation by the goblet cells. (d) *Test Methods:* Development of test methods and models for studies in chemical carcinogenesis continues. Studies of differences in host response due, for instance, to bacterial flora or the age and sex of the animal are undertaken for comparison with the situation in man.

Significance to Biomedical Research and the Program of the Institute: This group has consistently produced a high output of major research results in chemical carcinogenesis. They have provided considerable training in the field not very popular with a majority of investigators, partly because of the long-term nature of many of the experiments, and partly due to the frequency of negative results. With the move to the University of Nebraska, graduate students have been an added feature of the contract.

Proposed Course: The project will continue with the following three main objectives: (1) Bioassay activities on compounds of environmental significance. Approximately 75% of contract resources will be devoted to testing. Selection of compounds, experimental design, evaluation end points and criteria will be determined in strict collaboration with NCI. (2) Training of graduate students and postdoctoral scientists in the field of carcinogenesis, with particular emphasis in bioassay design and interpretation. (3) Research in carcinogenesis will focus on improved design and interpretation of bioassay, and on the understanding of the underlying biological mechanisms.

In summary, the contract will be a prime resource in the study, improvement and conduct of bioassay techniques in carcinogenesis. Procedures for clearer and more frequent communications with NCI have been established. A detailed cost accounting is being instituted. These measures will insure a satisfactory integration of the project with NCI activities and objectives.

Date Contract Initiated: March 19, 1968 (formerly Chicago Medical School--December 1, 1964)

Current Annual Level: $1,335,058

SUMMARY REPORT

BIOASSAY SEGMENT

Fiscal Year 1972

The Bioassay Segment, formally constituted in the summer of 1971, has for primary goal the evaluation of carcinogenic hazards in the environment through appropriate bioassays. In addition, the Segment considers the development, refinement and application of bioassay methods and additional studies required by the overall goal of the Segment.

In addition to the Director and the Manager of the Segment, there is an Advisory Group composed of three distinguished and experienced scientists from non-governmental institutions, one from the Food and Drug Administration, one from the Food and Drug Directorate of Canada, and six individuals from within other divisions of the National Cancer Institute. The Segment and its advisors have had two meetings to plan and formulate future activities and to monitor ongoing programs.

Continuing efforts have been given during the past year to standardizing the protocols for bioassays of environmental chemicals so that results in one laboratory will be comparable to those in others. Nine projects are now in effect using these standardized procedures for the bioassay of 148 compounds in approximately 80,000 animals. These chemicals include 37 pesticides, 31 drugs, 26 dyestuff intermediates and aromatic amines, 18 solvents, 24 chemical intermediates, and 6 chlorodibenzodioxins. Other contracts within the segment are designed to test industrial and environmental chemicals for carcinogenicity and co-carcinogenicity to the skin, the utilization of the pulmonary adenoma system in mice for identification of carcinogens, studies on carcinogenicity of fungal and plant products, and the effect of diet on chemical carcinogenesis.

A subgroup of the Bioassay Segment, under the chairmanship of Dr. L. Poirier of our staff, has examined the current status of short-term and in vitro tests; and their potential usefulness for the detection of carcinogenic potency in environmental chemicals. Thus considered were among others the utilization of newborn rodents and of immunosuppression. Of primary interest were the detection of carcinogenicity by in vitro cell or organ cultures and newer developments involving the application of mutagenesis in microbiologic systems as indicators. A report embodying the results of these deliberations is in preparation for formal publication.

The Bioassay Segment has proposed to allot a portion of its budget in the coming years to the accurate assessment of the utility of individual short term test systems as well as their judicious combination in order to detect carcinogenic hazards in a preliminary manner. It is visualized that with the development of a battery of such relatively simple and economic screens, applied in a preliminary fashion to a large number of agents which require evaluation, subsequent prolonged animal tests will be confirmatory and provide the authoritative information required to advise regulatory authorities and the public-at-large of potential risks. Also, the more lengthy full

scale animal studies would be utilized on agents positive in a prescreen and thus augment the chances for positive findings in such definitive studies.

Staff of the Bioassay Segment has assisted the Office of the Associate Scientific Director for Carcinogenesis to develop joint programs, workshops with other Government agencies such as the Food and Drug Administration dealing in part with similar problems and similar topics. Further direct contacts with other Government agencies such as the Environmental Protection Agency, The Institute for Occupational Safety and Health, and industrial organizations such as the pharmaceutical manufacturing association, and international groups such as the Japanese National Institute of Health, the Netherlands Public Health Institute, the International Agency for Research on Cancer in Lyon, and the like, are being established and maintained to coordinate efforts on a national and international scale.

Major accomplishments in the contract program include the following:

At Hazleton Laboratories studies are underway on the chronic toxicity and possible carcinogenicity of the most important pesticides and insecticides, particularly of the chlorinated hydrocarbon variety, to which man is exposed. Preliminary toxicologic data have been obtained and the definitive studies have been initiated. In a separate parallel program important chlorinated solvents and similar materials widely distributed in the environment are being studied under the same conditions.

At Bionetics Research Laboratory a number of important environmental chemicals have been examined. These studies are now reaching the end of their programmed test period. Two industrial chemicals, propane sultone and propylene imine, and one insecticide, ethylene thiourea, were found to be highly carcinogenic so far.

Bio-Research Consultants likewise has reached a terminal phase of a study dealing mainly with a number of monocyclic aromatic amines, important industrial intermediates and dyestuff components which have never been thus tested. It was found that several of them are carcinogenic in mice or rats.

Carcinogenicity studies at Southern Research Institute deal mainly with an examination of the properties of drugs used in cancer chemotherapy. These studies have documented that a number of these drugs, particularly alkylating agents such as Melphalan, uracil mustard, cytoxan, are also carcinogenic in mice and rats. The unexpected discovery was made that certain antimetabolites likewise have led to the development of tumors. The question whether the antimetabolites lead to cancer indirectly, perhaps by immunosuppression in the possible presence of a viral agent, such as polyoma, is currently under investigation.

Diet plays a role in determining toxicity and carcinogenicity of important environmental carcinogens such as mycotoxin and aflatoxin. Dr. Newberne at MIT is extending his early findings, that a low lipotrope diet simulating in part human foods in certain population groups decrease the acute toxicity but potentiate the carcinogenicity

of aflatoxin to other types of carcinogens with different organ specificities. Dr. Newberne also found that hamsters fed low levels of aflatoxin and deficient in vitamin A develop colon cancers which are absent in comparable controls on adequate vitamin A.

CONTRACT NARRATIVES

BIONETICS RESEARCH LABORATORIES (NIH-NCI-E-69-2085)

Title: Carcinogenicity of Chemicals Present in Man's Environment

Contractor's Project Director: Dr. Borge Ulland

Project Officer (NCI): Dr. John Weisburger

Objectives: To study the chronic toxicity and possible carcinogenicity in rats of a number of chemicals used in commerce and industry. Also to investigate the possible teratogenic effects of some of these environmental agents.

Major Findings: As reported last year in a preliminary way, two chemicals proposed for industrial development, propylene imine and propane sultone, are highly active carcinogens leading to tumors in rats in a number of tissues but interestingly produces glyomas in male and female rats and mammary cancer in female rats. Ethylene thiourea used as pesticide and in the rubber industry, among others, give a high yield of thyroid cancer. Preliminary information in some cases based on incomplete data suggest that the following chemicals were not carcinogenic: bis-(2-chloroethyl)-ether, bis-(2-hydroxy-ethyl)-dithiocarbamic acid, dithiooxamide, avadex, all of which were found active in an earlier study at Bionetics Research Laboratories in mice. Also inactive were sodium azide, ethylene carbonate, sodium sulfhydrate, thiosemi-carbazide, and semicarbazide hydrochloride. The positive control N-2-fluorenyl-acetamide led to expected number of types of tumors in male and female Charles River CD rats employed in this study. The antioxidant butylated hydroxytoluene (BHT), but not the antioxidant diphenyl-p-phenylenediamine, reduced tumor formation in the liver with the positive control compound N-2-fluorenylacetamide and its N-hydroxy metabolite. The underlying reason appears to be microsomal enzyme induction by BHT which leads to more extensive production and excretion of glucuronic acid derivative of N-hydroxy-N-2-fluorenylacetamide.

Even though propylene imine and propane sultone were highly active carcinogens when administered chronically to rats, they failed to induce cancer transplacentally. When pregnant female rats of the same strain were exposed in the latter part of their pregnancy the offspring kept lifetime had no excess cancer over controls. These two active carcinogens likewise exhibit little if any evidence of teratogenicity in rats.

Significance to Biomedical Research and the Program of the Institute: One primary effort in the prevention of cancer consists in determining potential carcinogenic hazards in the environment. The aim of this program is to

gather such data for a number of widely used materials. Additionally, the test procedures and the results obtained will be examined critically with the aim of improving the methodology by which such assays are conducted. Information on the teratogenic properties of these same chemicals will likewise assist the areas of preventive medicine, and simultaneously yield data of significance with respect to the problem whether the carcinogenic and teratogenic properties are related.

Proposed Course: Except for two experimental groups and corresponding controls all animals in this test series have come to autopsy. Histologic evaluation and data compilation remains to be done for some of the experiments. It is hoped that this entire program can be completed by the end of 1972.

Date Contract Initiated: June 28, 1968

Current Annual Level: $30,144

BIONETICS RESEARCH LABORATORIES (NIH-NCI-E-71-2146)

Title: Carcinogenesis Bioassay of Environmental Chemicals

Contractor's Project Director: Dr. Borge Ulland

Project Officer (NCI): Mr. Raymond Newell

Objectives: To assess in rats and mice the carcinogenicity and chronic toxicity of selected chemicals present in man's environment. Secondary objectives may deal with the pathogenesis of cancer induction.

Major Findings: Investigations relating to the determination of the maximal tolerated dosages of assigned chemicals in rats and mice are underway or have been completed. Two chronic studies have been initiated in rats.

Significance to Biomedical Research and the Program of the Institute: By identifying potentially hazardous substances present in the environment, using the rat as a model, it may be possible to minimize man's exposure by prohibiting or restricting the use of these agents or modifying the method in which they are processed and handled.

Proposed Course: Chemicals are fed to rats and mice over their life span at a maximally tolerated dose and one-half that level. All animals will be examined at death or at the end of the study for evidence of neoplasia and chronic toxicity.

Date Contract Initiated: May 13, 1971

Current Annual Level: $241,920

BIO-RESEARCH CONSULTANTS, INC. (NIH-NCI-E-68-1311)

Title: Carcinogenicity of Chemicals Present in Man's Environment

Contractor's Project Director: Dr. Freddy Homburger

Project Officer (NCI): Dr. John Weisburger

Objectives: The long-term effect of chronic administration of a number of chemicals to mice and rats is being evaluated.

Major Findings: Mice - Except for a few groups started late in the program many of the individual experiments have been terminated. In many instances, however, pathological evaluation of the autopsy material remains to be performed. It would appear on the basis of incomplete results that the large scale industrial chemical intermediates used in a variety of processes 2,4,5-trimethylaniline, 4-chloro-o-toluidine, 4,4'-methylenebis-(2-chloroaniline), 2,4,6-trichloroaniline may be carcinogenic.

Rats - Again preliminary data indicate that 2,4-diaminotoluene, o-toluidine, 4,4'-methylene-bis-(2-chloroaniline) and 2,5,-dimethoxy-4'-aminostilbene are carcinogenic leading to a variety of tumors depending on structure of the chemical.

Significance to Biomedical Research and the Program of the Institute: One primary effort in the prevention of cancer consists in determining potential carcinogenic hazards in the environment. The aim of this program is to gather such data for a number of widely used materials. Additionally, the test procedures and the results obtained will be examined critically with the aim of improving the methodology by which such assays are conducted. Information on the teratogenic properties of these same chemicals will likewise assist the areas of preventive medicine, and simultaneously yield data of significance with respect to the problem whether the carcinogenic and teratogenic properties are related.

Proposed Course: The remainder of the mice and rats still on test will come to autopsy in June 1972. Processing of the tissues and histological evaluation should be completed during Fiscal Year 1973.

Date Contract Initiated: June 26, 1968

Current Annual Level: $80,084

UNIVERSITY OF CALIFORNIA (SAN DIEGO) (NIH-NCI-E-70-2206)

Title: Pulmonary Tumors in Mice for Carcinogenic and Co-carcinogenic Bioassay

Contractor's Project Directors: Dr. Michael B. Shimkin
Dr. A. J. Kniazeff

Project Officer (NCI): Dr. Gio Gori

Objectives: The objectives develop along two lines: (1) *Bioassay* - Testing of chemicals for carcinogenic activity on the induction of pulmonary tumors in strain A mice. In 1940 Shimkin and Andervont developed a bioassay for chemical carcinogenesis based on the high susceptibility of strain A mice in developing adenomas. This system has been used during 30 years by several investigators, and appears to provide one of the fastest available tests for *in vivo* determinations. The test can be normally completed in 6-8 months and lately it has shown remarkable sensitivity and reproducibility. It has the advantage of not requiring histopathological examinations, as the counting of adenomas can be accurately made by gross necropsy. The principal investigator proposes to test a variety of chemicals (food additives from GRAS list, chemotherapeutic drugs, haloginated chemicals, carbomates, and related compounds and nitrosamines). Compounds already known to be positive or negative for carcinogenic activity will be retested in this system to determine to which degree the results parallel those of conventional bioassays of long term duration. Depending on the correlation of results, the usefulness of this test as a quick and inexpensive screening bioassay will be determined. Its value is obvious in view of the vast number of compounds that need to be screened and of the limited resources available.

(2) *Co-Carcinogenicity Investigations* - Little is known on the possible contribution of viral-chemical co-carcinogenesis in the pathogenesis of lung tumors. The proposed studies would attack this problem in two phases: (a) Evaluation of chemical-viral interaction on the production of pulmonary tumors in strain A mice, i.e. with selected viruses will enhance the tumorigenic response to administered chemical carcinogens. The viruses to be employed in these studies are Moloney leukemia and Moloney sarcoma viruses. The chemicals to be used are 3-methylcholanthrene and urethan. (b) To determine the suitability of monolayer cell cultures dried from trypsinized tissues and pretreated with viruses and chemicals for the detection of environmental compounds for carcinogenic activity. Cell types to be employed in these studies will be mouse lung and brain cells, whole embryo rat cells and human cells. After pretreatment with chemicals and viruses, cell populations will be monitored for transformation by such criteria as morphological changes, growth in soft agar, tumorigenicity, karyology, and growth characteristics to be measured by cell count and DNA synthesis. They will then be re-exposed to a chemical carcinogen and monitored as above to determine whether they exhibit characteristics of transformed cells. If they exhibit characteristics of transformed cells, they would then be considered suitable as substrates for testing of chemicals for carcinogenic activity.

Major Findings: (1) To date *in vivo* bioassay studies have been completed in 35 food additives. Of these, three appear to be positive in our system and current repeat studies are underway to confirm this. (2) In our experience mouse cell populations appear unsuitable for testing of chemicals for carcinogenic activity due to their *in vitro* instability. However, studies are underway to confirm this. Nevertheless, it was decided to employ whole embryo rat cell and human cell populations in the studies.

Significance to Biomedical Research and the Program of the Institute: This project will integrate in the activities of the Carcinogenesis Area by

contributing to outstanding program priorities as follows: (1) Promises to provide much needed rapid bioassay methods and testing for screening chemicals. Research for such methods is of first priority, due to the vast number of chemicals to be tested and the severe limitations on funds and resources. (2) It will provide timely extension of the existing bioassay capabilities.

Proposed Course: To continue as outlined above.

Date Contract Initiated: June 22, 1970

Current Annual Level: $147,831

UNIVERSITY OF CINCINNATI (NIH-NCI-E-64-45)

Title: Determination of the Carcinogenic and Co-Carcinogenic Properties of Industrial Chemicals

Contractor's Project Director: Dr. E. Bingham Mattheis

Project Officer (NCI): Dr. Richard Bates

Objectives: The purpose of this project is to test for skin carcinogenicity and co-carcinogenicity selected industrial chemicals to which there is significant human exposure.

Major Findings: A large portion of effort for the past year has been to determine the potential of optical brighteners to enhance the carcinogenic potency of ultraviolet light. The earlier observation that optical brighteners enhance the carcinogenic potency of ultraviolet light at 254 nm has been confirmed. At 290-320 nm, the carcinogenic wavelengths in sunlight, one brightener appeared to inhibit the carcinogenic response, two others were not significantly different from the usual potency at this wavelength. Preliminary data on one brightener suggests an enhancement of the carcinogenic potency at this wavelength. Additional experiments are underway to determine the extent of this effect. At 350 nm, a non-carcinogenic wavelength, it was observed that n-dodecane induced tumors in C3H mice. Experiments are underway to determine whether n-dodecane enhances the carcinogenic potency of ultraviolet light at 254 nm and 290-320 nm.

As of the recent epidemiological investigation that has demonstrated an increased risk of developing lung cancer among coke oven workers, an attempt is being made to determine the factors contributing to the potency of the effluent from coke ovens. Experiments at this laboratory demonstrate that benzo[*a*]pyrene is present in a high concentration in many (but not all) samples of the coal tar that is a by-product from a coke oven. However, other compounds, probably heterocyclic nitrogen compounds, also contribute to the carcinogenic potency of coal tar. Co-carcinogenic compounds are also suspected as contributing to the total potency. Samples of coke oven effluent have been collected. The benzene soluble fraction and certain other fractions are being prepared for determining carcinogenic potency by topical application to skin.

Significance to Biomedical Research and the Program of the Institute: The chemicals under investigation in this contract are found in the environment (home or occupational) of human beings. It is essential to know whether these materials are carcinogenic or markedly enhance (or inhibit) the potency of a "natural carcinogen", ultraviolet light. A most important function of this contract is to determine how combined exposure to various agents may affect their carcinogenicity. It has been demonstrated, for example, that chemicals which are not carcinogenic when tested by themselves may markedly enhance the carcinogenicity of other materials. This observation has very important implications for programs designed to restrict human exposure to carcinogens.

Proposed Course: The experiments described above will be completed and other experiments on the carcinogenic and co-carcinogenic potency of industrial chemicals will be started.

Date Contract Initiated: September 11, 1963

Current Annual Level: $75,459

COLUMBIA UNIVERSITY (NIH-NCI-E-67-731)

Title: Carcinogenicity of Fresh and Mildly Oxidized Vegetable and Animal Fats

Contractor's Project Director: Dr. Hans Kaunitz

Project Officer (NCI): Dr. Umberto Saffiotti

Objectives: This contract was initiated in an attempt to determine the effects on general health, growth rate, tumor incidence and other disease conditions of the feeding to rats of purified diets containing specific fresh and mildly oxidized fats.

Major Findings: The biological tests of several fresh and mildly oxidized vegetable and animal fats have been concluded. Differences in survival rate and tumor response were reported. The chemical analyses have suggested the presence of dimethylnitrosamine in some of the samples. This finding could be a key to explain the different biological activity of different fat samples that have been tested.

Significance to Biomedical Research and the Program of the Institute: Fats are major constituents in man's diet and the "safety" of the various dietary fats has been assumed as established. However, there is little information on the minor constituents of fats in the diet and their effects on carcinogenesis. This project, now terminated, was designed to explore the potential hazards of specific fats of plant and animal origin, and their oxidation products.

This contract fits into the overall program as a screening test on the carcinogenicity of dietary fat constituents.

Proposed Course: Termination

Date Contract Initiated: June 14, 1967

Current Annual Level: $49,871

GULF-SOUTH RESEARCH INSTITUTE (NIH-NCI-E-70-2210)

Title: Carcinogenesis Bioassay of Pesticides and Other Environmental Chemicals

Contractor's Project Director: Dr. Harry Burchfield

Project Officer (NCI): Dr. Richard Bates

Objectives: The objectives of this project are to test a number of pesticides for carcinogenicity in mice and rats by administration in the diet.

Major Findings: Technical grade compounds (18) to be used in the long-term feeding studies have been analyzed for purity using thin layer and gas chromatography, infrared and mass spectroscopy and other procedures. In addition, analytical standards (5) were analyzed in the same manner. Each new batch of test chemical will be analyzed in this same way. Procedures were developed for analysis of eighteen of the test chemicals in mixed feed and 117 feed samples were analyzed. Stability studies on some of the phosphate pesticides to determine storage stability in feeds were undertaken and suitable storage and mixing conditions determined. Feed analyses on 4 test chemicals were conducted to help in determining pesticide levels for the long-term feeding program. Endogenous residues of pesticides and PCB's in feed were determined on feeds from this laboratory and from other contractors in the program.

Synthesis of photodieldrin was accomplished and the product purified in sufficient quantities so that rangefinding studies have been made using this compound.

Rangefinding tests on 21 compounds, some more than once, were conducted with a total of 970 male and 1,005 female Osborne-Mendel randon-bred rats and 950 male and 980 C57Bl x C3H inbred mice. Chronic studies have begun with rats on 12 compounds (1 compound was restarted) using 1,430 male and 1,430 female rats. Chronic studies with 2 compounds have been started with a total of 220 male and 220 female mice. These figures include controls. During the year 2,400 male and 2,435 female rats and 1,170 male and 1,200 female mice have been used.

Significance to Biomedical Research and the Program of the Institute: Many of the pesticides under study have widespread usage resulting in extensive human exposure to them. The chemicals selected for testing have either been tested inadequately for carcinogenicity in the past, or the results of such tests have been equivocal. These extensive experiments are designed to yield definitive data and help resolve these issues.

Proposed Course: Long-term feed tests will continue and the remainder of the test chemicals will be placed on chronic studies in the near future. Chemical analysis of each new batch of test chemical will be made and analysis of feeds for test chemical will continue as a check on the mixing procedure. Analysis of feed samples for endogenous pesticides and PCB's from this laboratory and the other contractors will continue. Synthesis of photodieldrin for use in the long-term feeding tests will continue.

Date Contract Initiated: June 15, 1970

Current Annual Level: $293,074

HOWARD UNIVERSITY (NIH-NCI-E-71-2167)

Title: Chemical and Biological Investigation of Potential Carcinogens from Plants

Contractor's Project Director: Dr. Govind Kapadia

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: (1) To prepare purified fractions from certain herbs that are used habitually in Curacao and South Carolina and isolate and characterize crystalline compounds from these fractions, (2) to test each fraction and compound for carcinogenicity in experimental animals and identify substances responsible for the carcinogenic effects, (3) to extend such chemical and bioassay studies to plant species related to the above as well as to other plants that are suspected of possessing carcinogenic activity and (4) to elucidate the chemical structure of any carcinogenic substance that may be isolated.

Major Findings: There is a high frequency of esophageal cancer in the natives of Curacao and the Gullahs' of South Carolina that is possibly related to the ingestion of unknown carcinogens of plant origin. Certain herbs used as folk remedies are especially suspect. In earlier studies, Dr. O'Gara, NCI, observed an elevated tumor incidence in rats on administration of crude plant material as well as concentrated extracts of *Krameria*, *Acacia*, and *Annona* that were prepared by Dr. Kapadia. Additional suspect plant materials from Curacao as well as South Carolina are now being tested at Howard University for carcinogenic activity using the NIH black rat.

Significance to Biomedical Research and the Program of the Institute: This project is directly related to the major objective of the Carcinogenesis Program, i.e., the identification of unknown carcinogens in natural plant materials. This project should serve as the ultimate check to ascertain the carcinogenicity of materials implicated by epidemiological studies on the island of Curacao. Information gained from this study could conceivably lead to definitive studies that should be conducted in other high incidence areas to identify the carcinogens involved.

Proposed Course: This contract is complimentary to the University of Miami, Contract No. NIH-NCI-E-71-2274 which provides for the collection of the plants in Curacao and South Carolina. It is estimated that this contract will extend for two additional years.

Date Contract Initiated: June 25, 1971

Current Annual Level: $75,083

IIT Research Institute (NIH-NCI-E-71-2338)

Title: Carcinogenesis Bioassay of Chlorinated Dibenzodioxins and Related Chemicals

Contractor's Project Director: Dr. Maurice King

Project Officer (NCI): Dr. Richard Bates

Objectives: To determine the carcinogenicity and co-carcinogenicity of chlorinated dibenzodioxins by application to the skin of mice and by oral administration to rats and mice.

Major Findings: Trial syntheses of a series of chlorinated dibenzodioxins has been carried out. The synthesized compounds have been analyzed for purity by a combination of gas chromatography and mass spectrometry. Experiments to determine the carcinogenicity and carcinogenesis promoting activity of these materials by application to the skin of mice have been initiated. After 12 weeks of application neither the dichloro-, the octachloro-, nor the unsubstituted dibenzodioxin have produced any abnormal skin changes indicative of skin tumor formation. However, promotion studies in mice pretreated with DMBA followed by treatment with dioxane or croton oil have yielded papillomas after seven weeks of application.

In order to determine the frequency with which oral administration of the chlorinated dibenzodioxins should be carried out, high activity Cl^{36}-octachloro-dibenzodioxin was prepared by neutron irradiation in the Argonne National Laboratory reactor. Only a small fraction of material given to rats by oral intubation was absorbed. Low levels of activity were found in serum and liver of serially sacrificed animals. Serum activity was maximal one hour after intubation, dropped sharply at two hours, then gradually decreased during the remainder of the 72-hour observation period. Liver levels of Cl^{36} reached a maximum at 24 hours and fell gradually for the remainder of the observation period. This study will be repeated and similar studies with Cl^{36}-hexachlorodibenzodioxin, a more toxic member of this series, will be carried out. Large scale synthesis of the compounds of interest is now underway and carcinogenicity trials by oral administration will be initiated in the near future.

Significance to Biomedical Research and the Program of the Institute: Some chlorinated dibenzodioxins are extraordinarily toxic contaminants in certain pesticides and other environmental chemicals. In addition to their extreme

toxicity, they are highly stable under environmental conditions and tend to accumulate in the food chain. They have been tested for acute toxicity and teratogenicity but have not been examined for carcinogenicity. This program is designed to accomplish the latter and is part of the NCI program of testing chemicals to which man is exposed for carcinogenicity.

Proposed Course: This study is expected to extend over three-four years.

Date Contract Initiated: June 25, 1971

Current Annual Level: $320,000

MASON RESEARCH INSTITUTE (NIH-NCI-E-71-2144)

Title: Carcinogenesis Bioassay of Environmental Chemicals

Contractor's Project Director: Dr. Marcus Mason

Project Officer (NCI): Mr. Raymond Newell

Objectives: To study the chronic toxicity and possible carcinogenicity of environmental chemicals.

Major Findings: Studies relating to the determination of the maximum tolerated doses of assigned chemicals in rats and mice are underway. This is the first year of the project and no major findings have been obtained.

Significance to Biomedical Research and the Program of the Institute: One of the functions of the Carcinogenesis Program is to evaluate the potential carcinogenic risk that is represented by chronic exposure to environmental chemicals. This contract will be significant in identifying and minimizing potentially hazardous substances to which man is exposed.

Proposed Course: In this study rats and mice will be fed chemicals at a maximally tolerated dose and one-half that over a two-year period. The overall project is expected to continue until approximately 1974.

Date Contract Initiated: May 1, 1971

Current Annual Level: $213,550

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NIH-NCI-E-62-468)

Title: Toxicity and Carcinogenicity Associated with Fungal Growth on Foodstuffs

Contractor's Project Directors: Dr. Gerald Wogan
Dr. George Büchi
Dr. Arnold Demain

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: To investigate the biochemical, toxic and carcinogenic effects of aflatoxins, their metabolites and analogs; to determine the metabolic fate of aflatoxins in various species; to synthesize aflatoxin metabolites; and to isolate, characterize and investigate the toxicity and carcinogenicity of other mycotoxins produced by molds isolated from foods or food raw materials.

Major Findings: Carcinogenesis evaluations on dose-response relationships of aflatoxin B_1 and on comparative potency of B_1 and M_1 (hydroxy-B_1) in rats are still in progress. A laboratory method for converting B_1 into P_1 (the principal urinary metabolite) has been developed, and sufficient P_1 has been produced to permit initiation of studies of its toxicity and carcinogenic activity. Metabolites of aflatoxin B_1 are being isolated from mouse urine in order to make qualitative and quantitative comparisons with other more susceptible species. Further progress has been made in isolation and toxicological evaluations of a potent mycotoxin produced by *Aspergillus glaucus*. Fermentation techniques are being established for production of that toxin as well as for initial studies on approximately 30 additional toxin-producing fungi isolated from Asian foodstuffs.

Significance to Biomedical Research and the Program of the Institute: Evidence is accumulating that chemical products arising from microbial spoilage (particularly involving mold-damage) of foods or food raw materials can appear in the human food supply and may be associated with the etiology of liver cancer in several areas of Asia and Africa. The scope of the problem in terms of numbers of compounds and/or molds that may be involved has not yet been accurately defined, but is potentially great. This project, which is related to other NCI contracts on naturally-occurring carcinogens, is designed to produce relevant information on these questions.

Proposed Course: Experiments will continue along the lines suggested by the objectives outlined above. Studies on the comparable metabolism of aflatoxins may help to delineate the mechanisms involved in rather wide species differences in response to these compounds. Similarly, studies on the biochemical effects, toxicity and carcinogenicity of analogs and metabolites may provide evidence on active forms of the agent, and on sites of action. Identification of new mycotoxins from other molds isolated from food samples will provide further definition of the scope and character of the potential hazards to man represented by these toxins.

Date Contract Initiated: June 25, 1962

Current Annual Level: $173,500

UNIVERSITY OF NEBRASKA (EPPLEY INSTITUTE FOR RESEARCH ON CANCER)
(NIH-NCI-E-68-959)

Title: A Resource for Carcinogenesis Bioassays and Related Research

Contractor's Project Director: Dr. Philippe Shubik

Project Officer (NCI): Dr. Gio Gori

Contract narrative is reported under the Summary Report for the Office of the Associate Scientific Director.

SOUTHERN RESEARCH INSTITUTE (NIH-NCI-E-68-998)

Title: Carcinogenicity of Chemotherapeutic Agents

Contractor's Project Director: Dr. D. P. Griswold

Project Officer (NCI): Dr. John Weisburger

Objectives: To evaluate the potential carcinogenicity of chemotherapeutic agents and related chemicals in rats and mice following long-term exposure.

Major Findings: Preliminary histopathological evidence indicates increased carcinogenic activity (>50% over control values) in rats or mice chronically exposed to the following 16/26 compounds thus far evaluated: actinomycin D, mitomycin C, 6-mercaptopurine, azathioprine, Cytoxan, DIC, BIC, melphalan, uracil mustard, procarbazine, o-merphalan, CCNU, BCNU, phosphorodiamidic acid, N,N-bis(2-chloroethyl)-N'-3-hydroxypropyl cyclohexylamine salt, 1-acetyl-2-picolinoylhydrazine and mixture G (actinomycin D, chlorambucil, and methotrexate).

Significance to Biomedical Research and the Program of the Institute: Results will provide clinicians with a basis for the selection of agents to be used in long-term chemotherapy in man.

Proposed Course: To continue assaying the carcinogenic potential of those agents to which man is currently exposed or may be exposed for extended periods of time. Those compounds showing an increased tumorigenesis will be further evaluated for carcinogenic potential at various lengths of exposure.

Date Contract Initiated: June 24, 1968

Current Annual Level: $291,790

TRW-HAZLETON LABORATORIES (NIH-NCI-E-70-2209)

Title: Carcinogenesis Bioassay of Pesticides and Other Environmental Chemicals

Contractor's Project Director: Dr. W. M. Weatherholtz

Project Officer (NCI): Dr. John Weisburger

Objectives: To determine the carcinogenicity of selected environmental chemicals administered chronically to rats and mice at the maximum tolerated dosage.

Major Findings: This program dealing with the chronic toxicity of important extensively used environmental chemicals, especially pesticides, in Osborne-Mendel rats and C57BL x C3H mice is currently in its initial stages. After performing purity tests on all chemicals used and evaluation of a maximally tolerated dose of each chemical in the bioassay systems used here, definitive studies have been initiated. At this time all bioassays are underway in rats and the mouse studies are being phased in at the present time.

Significance to Biomedical Research and the Program of the Institute: Because of extensive use, pesticides and related compounds are ubiquitous. Some of them have accumulated in man. Their involvement in the causation of human disease is virtually unknown. The purpose of this program is to gather fundamental data so that adverse effects in man can be predicted, and hopefully prevented.

Proposed Course: After phasing in the remainder of the mouse studies, administration of test chemicals will continue in effect for a total period of 18 months. After that time groups of mice will be held at least three more months and groups of rats six more months at which time autopsies and histological analysis of the tissues will be undertaken.

Date Contract Initiated: December 1, 1970

Current Annual Level: $347,774

TRW-HAZLETON LABORATORIES (NIH-NCI-E-71-2145)

Title: Carcinogenesis Bioassay of Environmental Chemicals

Contractor's Project Director: Dr. W. Weatherholtz

Project Officer (NCI): Mr. Raymond Newell

Objectives: To determine the carcinogenicity of selected environmental chemicals administered chronically to rats and mice at the maximum tolerated dosage.

Major Findings: Initiation of all rat subacute studies were performed to determine the maximum tolerated dosage. The importance of establishing the maximum tolerated dosage has necessitated repeating 50% of the subacute studies with adjusted dosage levels. One chronic rat study has been initiated. Eight mouse subacute studies have been initiated but at a slower rate than the rat studies due to slower availability of mice. Chemical analysis of seven test materials were completed during this first contract year.

Significance to Biomedical Research and the Program of the Institute: One of the functions of the Carcinogenesis Program of the NCI is to evaluate the potential carcinogenic risk which is represented by chronic exposure to environmental chemicals. This is one of three major contracts awarded in 1971 for this purpose.

Proposed Course: Subacute studies in both species will be completed. Air sampling to monitor the presence of hazardous volatile test chemicals will be initiated in the animal-holding rooms. Chemical analysis of all test materials to determine the identification and purity will be completed to coincide with the initiation of the chronic studies at the maximum tolerated dosage.

Date Contract Initiated: May 1, 1971

Current Annual Level: $189,900

TEMPLE UNIVERSITY (NIH-NCI-E-65-1029)

Title: A Search for Carcinogens among Mycotoxins

Contractor's Project Director: Dr. Fritz Blank

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: This program is aimed at identification of possible carcinogens from fungi pathogenic to man. The original screening program included various extracts and fractions from 17 fungal species. Presently, efforts are being concentrated on *Candida parapsilosis* and *Candida albicans*, the most often isolated pathogenic fungus from clinical specimens and *Scopulariopsis brevicaulis* because these species appeared to have the highest carcinogenicity in the preliminary screening program.

Major Findings: Mycelia of the fungi are produced and lyophilized (Dr. Blank). Extraction of the mycelia (hexane, chloroform, hot and cold methanol) and fractionation of these extracts for bioassay is performed by Dr. G. Just, McGill University. Bioassays are done by Dr. S. J. Mann at Temple University. In strain A mice, injection of a high dose of the hot methanol extract of *Candida parapsilosis* gave a significant increase in frequency of lung tumors. A scheme of separation of the major fractions of *Candida parapsilosis* has been developed.

Significance to Biomedical Research and the Program of the Institute: This study deals with potential carcinogenic fungal metabolites which may be of importance to man through contact or their presence in food or other commodities. Such metabolites may be responsible for some of the "spontaneous" cancer incidence in the population.

This program is related to the other mycotoxin studies at Massachusetts Institute of Technology (NIH-NCI-E-65-1036) which are funded by NCI.

Proposed Course: The largest number of sarcomas was induced by certain extracts of *Candida parapsilosis*. When repeated, fewer sarcomas were obtained as a result of changed growth conditions: the original method of growing was in small Erlenmeyer flasks whereas the later production was done in a fermentor for reasons of economy. Experiments are underway for production of the fungus under the original conditions. At the same time, the co-carcinogenicity of the *Candida parapsilosis* extracts is being investigated.

Date Contract Initiated: June 12, 1965

Current Annual Level: $87,369

SUMMARY REPORT

BIOLOGICAL MODELS SEGMENT

Fiscal Year 1972

The Biological Models Segment was formed this year with the charge of establishing a program leading to the development of useful experimental biological models of the major types of cancers observed in man. Lung cancer and cancer of the colon and rectum are being investigated in other segments organized specifically for this purpose.

The Segment now consists of a Director, a Manager, four NCI scientists, and seven scientific advisors from academic and research institutions. The advisors were chosen for their general knowledge of carcinogenesis or for a specific interest and capability with individual target organs.

One of the first decisions of the Segment was to put special emphasis this first year on cancers of the following organs: (1) pancreas, (2) endocrine related tissues such as prostate and breast, (3) kidney and urinary bladder, and (4) organs particularly susceptible to cancer in childhood. Particular activity during this fiscal year was generated in initiating programs in carcinogenesis of the pancreas and prostate. It is anticipated that between seven and ten contracts will be initiated in these areas by the end of the fiscal year. In addition, one new and one old contract deal with mammary carcinogenesis, one new contract on renal carcinogenesis and one on transplacental carcinogenesis are being established. A previously established contract on liver carcinogenesis is continuing. A former contract on the carcinogenesis of metallic compounds is now placing considerable emphasis on the roles of cadmium and zinc in the development of tumors of the prostate and testis. Other contracts within the Segment are designed to define the sensitivity of bioassay systems in order to study the influence of combinations of chemicals on the carcinogenicity of individual components of the mixture.

Plans for the next fiscal year call for particular emphasis on development of programs for the urinary bladder and childhood tumors with an expansion of the other programs begun this year, consistent with personnel and budgetary resources made available to the Segment.

Other activities of the year include the nationwide promulgation of our specific interest in cancer of the pancreas by submission of over 200 letters to the membership of a pancreas society recently established. In addition, capability statements of over 100 investigators responding to notices in Science, Cancer Research, etc. have been abstracted and placed in the DCRT computer for retrieval as desired for solicitation of research proposals.

ALTON OCHSNER MEDICAL FOUNDATION (NIH-NCI-E-71-2131)

Title: Carcinogenesis by Radiation Plus Estrogen

Contractor's Project Director: Dr. Albert Segaloff

Project Officers (NCI): Dr. Norbert Page
Dr. Jane Taylor

Objectives: (1) To investigate interaction or synergism of radiation and estrogens in the induction of mammary tumors. (2) To develop a small animal model to simulate the human menstrual cycle.

Major Findings: A preliminary study indicated that a single 800 R Dose of X-rays delivered to one mammary chain of AXC inbred rats which had also received a continuous, exogenous source of diethylstilbesterol resulted in a significant increase in mammary tumors over that resulting from estrogen or irradiation alone. In addition the first tumors appeared earlier in those animals receiving both X-ray and estrogen (Cancer Res. 31: 166-168, 1971).

During the past year studies were initiated with lower doses of x-radiation (50, 150, and 450 R) using the same level of estrogen that induced tumors in the preliminary study. Tumors have already appeared in all radiation and estrogen combination treatment groups, while all other control groups are negative. Thus, the synergistic action appears to exist at the relatively low dose of 50 R.

Attempts are underway to develop a small animal model that can simulate the human menstrual cycle using spayed rats and variable doses of exogenous estrogens. Only partial success has been achieved from this endeavor. While it has been possible to produce a regular cycle for a couple of months, the animals progressed into a constant estrus similar to that observed with the estrogen implants. More research will be devoted to this aspect during the coming year.

Significance to Biomedical Research and the Program of the Institute: Both radiation and ovarian hormones, given individually in large doses have been implicated in causing an increase in breast cancer of both laboratory animals and women. Doctor Segaloff and colleagues have speculated that X-rays, routinely used for diagnosis and radiotherapy, plus estrogens and progesterones, now being widely used in birth control pills or for postmenopausal therapy, may interact synergistically and be contributing to the alarming rise in the incidence of breast cancer.

In recent years the Carcinogenesis Program of NCI has devoted some efforts and funds to studies of the hormones, progestogens and estrogens, which are often combined in contraceptive pills. This study is an extension of efforts in this field. In addition, the project would involve studies of the possible role of viruses in neoplasms induced with X-irradiation and estrogens. Therefore, this program is of interest to the Breast Cancer Task Force and the Special Virus Cancer Program.

Proposed Course: The radiation/estrogen studies now underway are expected to be completed by mid-1974. It is expected that additional studies will be initiated to vary dosages of estrogen administered by various routes which would mimic more closely the estrogen content in the human female contraceptives.

It is also planned to continue research to set up an artificial estrus cycle of the same length as the natural cycle of the rat. The point of major importance here would be to determine the dosage of estrogen (Estradial, 17B, ethynylestradiol, etc.) necessary to duplicate the natural estrus cycle in order to then determine precisely the quantity of estrogens required (with the additional amount found in the contraceptives) to influence the induction of mammary tumors in combination with a known dosage of X-ray.

With the completion of the initial experiments and the establishment of an artificial estrus cycle developed as discussed above, it is proposed to cycle the estrogen therapy and retain the radiation dose and quality that also has been established as effective. It is important here to determine if estrogen induction of breast cancer can be demonstrated in the absence of the host's ovaries. All other studies have been done in the intact host.

The final experiment, as now projected, would be to extend the radiation-estrogen work to the Fischer/344 strain of inbred rats. With data from the other studies as outlined above it should be possible to more rapidly determine the carcinogenesis by radiation and estrogen in this inbred strain of rats whose incidence of spontaneous mammary tumors is "high risk" in comparison to the "low risk" of the AXC rats. This is of interest because of the data of the human disease showing high and low risk populations.

Date Contract Initiated: May 25, 1971

Current Annual Level: $ 74,150

BIONETICS RESEARCH LABORATORY (NIH-NCI-E-72-2063)

Title: Preparation and Examination of Experimental Biological Material

Contractor's Project Director: Dr. Elizabeth Kingsbury

Project Officer (NCI): Dr. Curtis Harris

Objectives: The principal objective is the preparation and examination of tissues for light and electron microscopy, in collaboration with the NCI collaborative research program, studying the following:

1. the pathogenesis of chemically induced tumors of various organs such as prostate, pancreas and lung, and
2. the autoradiographic localization of labelled carcinogens or other relevant biological compounds, such as Vitamin A.

Major Findings: Since this project was initiated December 1, 1971, the necessary personnel and equipment have been obtained, and several studies are presently being conducted. The detection and characterization of a C-type virus in tissue cultures derived from DEAE-dextran induced tumors is in progress. The study of the morphology of the developing prostate in mice has been initiated. Specimens are being processed for autoradiography to determine the localization of vitamin A in the prostate and pancreas of normal and vitamin A deficient hamsters. The normal ultrastructure of the exocrine pancreas in guinea pigs, mice and hamsters has been studied for future comparisons during pancreatic carcinogenesis.

Significance to Biomedical Research and the Program of the Institute: Chemical carcinogens are becoming increasingly widespread throughout man's environment. In order to protect humans from the carcinogenic effects and to decrease his susceptibility to such chemicals, data must be gathered concerning the following:

1. the mode of action of these carcinogens in various organs,
2. the susceptibility states of various tissues, and
3. the development of models for the study of specific organ carcinogenesis.

Proposed Course: Continuation of the studies outlined above.

Date Contract Initiated: December 1, 1971

Current Annual Level: $ 147,708

UNIVERSITY OF CHICAGO (NIH-NCI-E-69-2087)

Title: Definition of Sensitivity of Carcinogenesis Bioassay Systems

Contractor's Project Director: Dr. Stan Vesselinovitch

Project Officer (NCI): Dr. Jerry Rice

Objectives: To establish standard bioassay systems in mice for screening substances suspected of potential carcinogenic hazard to man, making best use of the age-dependent variation in sensitivity to chemical carcinogens exhibited by F-1 hybrid mice (first generation crosses of C3HeB x A/He and C57Bl/6 x C3HeB) and to provide definition of diagnostic criteria and of detailed biologic behavior of the neoplasms produced, especially those of the liver and lung.

Major Findings: Untreated control animals of both strains have now been examined for spontaneous tumor incidence through the age of 90 weeks. The spontaneous incidence of tumors of the lung rises very rapidly to a significant incidence in the C3HeB x A/He hybrid between 60 and 90 weeks of age, suggesting that it is of less potential value as a routine screening animal than the alternative hybrid, in spite of its somewhat greater sensitivity to the chemical induction of tumors of the gastrointestinal tract and the central nervous system. The spontaneous incidence of other types of tumors has been

extremely low in both strains, and no non-neoplastic, chronic disease has been identified in either strain which might preclude their use in bioassay programs. A striking finding which emphasized the critical importance of the neonatal age period to the detection of carcinogenic agents is the recent demonstration that mice of the C57Bl/6 x C3HeB hybrid develop a very high incidence of liver tumors as a consequence of exposure to aflatoxin B-1 given during the first week of life. This substance, which is powerfully carcinogenic to the rat and suspected of possible etiologic significance to man, has previously been found to be at best marginally carcinogenic when fed for very prolonged periods of time to adult mice of several strains. This adds weight to previous suggestions that efforts should be made to develop regular testing protocols in which the much greater sensitivity of neonatal animals to chemical carcinogens would be utilized. Transplantation of hepatocellular tumors arising in mice treated at different intervals during life with chemicals that are either known powerful carcinogens, or weaker agents of known or suspected hazard to man, have been begun. Preliminary results indicate that tumors which in situ display no indication of invasiveness or metastasis, nevertheless on transplantation to syngeneic recipients grow progressively, invade surrounding tissues aggressively, and readily metastasize via the lymphatics to the lung. This emphasizes earlier suspicions that histology alone provides an insufficient criterion for assessing the significance of hepatocellular tumors of the mouse in carcinogenesis bioassay programs.

Significance to Biomedical Research and the Program of the Institute: A major deficiency in carcinogen screening programs has been their lack of uniformity. It has been in the past quite impossible to estimate the relative carcinogenic potency of different compounds, even those tested in the same species, because of uncontrolled variables associated with testing protocols. These include the intrinsic differences in neoplastic response of different tissues in various strains of mice, the pronounced quantitative differences in response of fetuses, infants, and adults, and the marked effects of route of administration and dosage regimen. The dissimilarity between the histological features of murine versus human tumors of corresponding cell type has also led to disagreements, which require resolution, concerning the significance of certain neoplasms of the mouse to assissment of cancer risk in man.

The purpose of this contract is to evaluate the significance of these variables within the context of an optimal screening protocol, and to develop improved standardized procedures which will be used for future carcinogen screening programs. Definition of biologic behavior and diagnostic criteria for the histologic identification of the neoplasms that can be induced in these animals will provide needed reference standards for the evaluation of endpoints in carcinogenesis tests.

Proposed Course: Studies currently underway in both strains of mice will be continued with particular reference to identification of any susceptibility to chronic neoplastic or non-neoplastic disease, which might preclude the satisfactory use of either strain of mouse in carcinogenesis screening programs. Future studies will be directed towards the utilization of the greater sensitivity of neonatal animals in developing model bioassay protocols. Studies currently underway will be continued to specify clearly the biological nature of mouse hepatocellular tumors as a function of the specific chemical inducer,

the age of the animal at the time treatment was begun, and the age of the animal at the time the tumor appeared.

Date Contract Initiated: June 25, 1969

Current Annual Level: $ 390,000

MICROBIOLOGICAL ASSOCIATES (NIH-NCI-E-70-2199)

Title: Laboratory Service for Support in Carcinogenesis Bioassay and Related Activities

Contractor's Project Director: Dr. Russell Madison

Project Officer (NCI): Dr. Jerry Rice

Objectives: This contract provides for laboratory support services for the Carcinogenesis Area, NCI, in the field of experimental pathology. The project is intended to provide the following services under the direct supervision of the NCI staff: (1) perform bioassay studies according to protocols developed especially by NCI, including controlled feeding, parenteral administration, skin painting, intragastric intubation, intratracheal instillation and other techniques as required, using mice, hamsters and rats; (2) perform animal treatments and maintain precise observation records as prescribed by individual NCI staff members who have initiated the protocols for different types of carcinogenesis studies; (3) conduct gross and histopathological examinations and evaluations of experimental animals according to NCI specifications, and to provide histological support for NCI in-house activities; (4) provide photographic services as required by the project and other NCI needs.

Major Findings: During the second year of the contract, both facilities have functioned at full capacity, with a rapid turnover among projects, of which approximately 20 are in progress at any given time. These protocols were initiated by 10 different investigators within the Carcinogenesis Area and have involved feeding studies, intratracheal instillation methods, and parenteral injections. The current animal inventory includes 1400 rats, 1800 mice, 800 hamsters, less than 100 guinea pigs. Twenty nine experiments were completed during the current year. The histology laboratory has completed approximately 15,000 stained sections from experiments originating both in this contract facility and also in other programs of the Experimental Pathology Branch. The major areas of activity include the following: (1) intratracheal instillations of carcinogens and various forms of dust in hamsters, and transplantation of the tumors which develop, in support of studies carried out by the Lung Cancer Unit, OASDC; (2) preparation by the newly established electron microscopic preparation laboratory of large numbers of ultra-thin sections for histogenesis studies; (3) oral administration of carcinogens and related non-carcinogenic compounds in normal, protein deficient, and immune suppressed rats, in support of studies carried out by the Carcinogen Screening Section, Experimental Pathology Branch, and the Nucleic

Acid Section, Chemistry Branch; (4) holding experiments for rats injected with cells grown and treated with chemical carcinogens in tissue culture; and (5) parenteral administration of compounds, both carcinogenic and non-carcinogenic for the exploration of various aspects of chemical carcinogenesis by the Bioassay Section, Experimental Pathology Branch.

Significance to Biomedical Research and the Program of the Institute: This contract provides an essential facility for the initiation and completion of long-term experiments involving large numbers of experimental animals. As an extension of NCI in-house research, the facility provides a means for rapid testing of new ideas in experimental pathology related to chemical carcinogenesis.

Proposed Course: This contract should be renewed and greatly expanded in magnitude, in order to accommodate the large number of studies which are currently tabled pending availability of additional rooms in this facility. The scope of activities should be broadened to include laboratory work in support of live animal experimentation, including biochemical procedure and tissue culture techniques and facilities.

Date Contract Initiated: June 4, 1970

Current Annual Level: $333,000

UNIVERSITY OF NEBRASKA, (EPPLEY INSTITUTE FOR RESEARCH ON CANCER) (NIH-NCI-E-68-959)

Title: A Resource for Carcinogenesis Bioassays and Related Research

Contractor's Project Director: Dr. Philippe Shubik

Project Officer (NCI): Dr. Gio Gori

Contract narrative is reported under the Summary Report for the Office of the Associate Scientific Director for Carcinogenesis.

UNIVERSITY OF SAN FRANCISCO (NIH-NCI-E-64-886)

Title: Studies of Carcinogenicity of Metallo-Organic Compounds

Contractor's Project Director: Dr. Arthur Furst

Project Officer (NCI): Dr. Richard Bates

Objectives: This contract was designed to test metals and their compounds especially metallo-organic derivatives for their potential carcinogenicity. Different routes of administration are being investigated in various species of rodents. Studies on excretion of these compounds are also being conducted; this information may shed light on the mechanisms by which these metals cause cancer.

Major Findings: A few metals and compounds of these metals were shown to produce cancer in rodents by the intramuscular route, under the conditions of the experiment. Notably, all compounds of nickel tested except the oxide induced fibrosarcomas which metastasized to the lungs. These tumors were readily transplanted. Cadmium metal also produced fibrosarcomas at the site; the latent time for this metal was unusually short. Chromium oxide has border-line activity; however, an osteogenic sarcoma was found in the lungs of a rat. This experiment is being repeated. The lymphogenic activity of titanocene has been verified. This research aspect is being extended to other organo-titanium compounds.

Comparisons of routes of administration are underway, but it is too soon for results.

Significance to Biomedical Research and the Program of the Institute: The use of non-ferrous metals is continuously expanding in our industrial society. Metal refining industries are increasing in size and scope, as is the plating industry. Cadmium metal is being used in much greater quantities. Newer titanium catalysts are being developed for the polymerization process in the plastics industry. Select groups of workers are being exposed to these metals; also, the pollution of air and water by heavy metals is greater than ever before. It is necessary to determine which of these metals pose a human cancer hazard.

Proposed Course: The contractor is a major resource to the Carcinogenesis Program as an expert on the industrial usage of metals and their carcinogenicity. He will continue long-term studies along the lines outlined here of those metals to which humans receive significant exposure.

Date Contract Initiated: June 3, 1964

Current Annual Level: $ 104,406

STANFORD RESEARCH INSTITUTE (NIH-NCI-E-71-2166)

Title: Combined Effects of Chemical Carcinogens and Other Chemicals

Contractor's Project Director: Dr. David Jones

Project Officer (NCI): Dr. Richard Bates

Objectives: To investigate (1) the effect of mixtures of carcinogenic and non-carcinogenic chemicals on the induction of tumors when introduced into rodents by oral administration, (2) whether the interactions among groups of chemicals in acute and subacute toxicity experiments predict the effects of the same combinations in carcinogenicity experiments, and (3) the feasibility of conducting initial screening bioassays of chemicals in groups without thereby obscuring the carcinogenicity of any individual member of the group.

Major Findings: Thirteen compounds have been identified for testing singly and in certain specified pairs. Data on chemical identity, purity, and stability in diet have been collected for seven compounds. Using the Fischer 344 rat (both male and female), single dose intubation LD(50) and two-week acute toxicity feeding studies have been completed for 15 single or paired compounds at various dietary concentrations. From the mortality, food consumption, and body weight changes observed during the acute studies have been selected; the latter studies are now in progress. Procedures for selecting dietary levels to be used in chronic (lifespan) studies have been established.

Significance to Biomedical Research and the Program of the Institute: A major portion of the resources of the Carcinogenesis Area of the National Cancer Institute is devoted to testing environmental chemicals for carcinogenicity. In these tests, the experimental protocols generally attempt to expose animals to one chemical while minimizing exposure to other chemicals. There are two problems involved in this approach. First, in the human environment, men are exposed to multiple chemicals simultaneously and sequentially rather than to a single chemical. Second, resources are available to test only a small portion of the chemicals in our environment if they are tested singly. This project is the beginning of a program that will investigate how mixtures of carcinogenic and non-carcinogenic chemicals interact to affect the induction of tumors and also to investigate the feasibility of conducting initial screening bioassays of chemicals in groups without thereby obscuring the carcinogenicity of any individual member of the group. The biological studies from this contract will be analyzed by mathematical statisticians working on a contract with the Demography Area of NCI to determine whether consistent mathematical models of interactions can be developed.

Proposed Course: It is estimated that this project will extend over four years. Acute and subacute studies of individual chemicals and pairs of chemicals will be conducted in order to determine the dosage levels to be administered in the chronic carcinogenic studies. The results of all three studies will be analyzed statistically in order to determine what extent the interactions of two chemicals in carcinogenesis parallel the interaction seen in the short-term acute and subacute studies.

Date Contract Initiated: June 24, 1971

Current Annual Level: $ 613,339

TEMPLE UNIVERSITY (NIH-NCI-E-64-504)

Title: Research on the Biochemical and Morphological Components of Hepatic Carcinogenesis

Contractor's Project Director: Dr. Emmanuel Farber

Project Officer (NCI): Dr. Michael Sporn

Objectives: To develop a bioassay technique for rapid screening of potential

hepatocarcinogens using the hyperplastic nodule as the end-point; to establish the relationship of nodular hyperplasia to the origin of hepatocellular neoplasms; to characterize biochemically and morphologically subcellular markers of the hyperplastic nodule and determine their occurrence in nodules produced with diverse carcinogens; and obtain tissue in larger amounts for these studies. The test system should thus provide a means for rapid identification of hepatic carcinogens and offer insight into molecular mechanisms of pathogenesis.

Known or suspected hepato-carcinogens and hepato-toxins are fet to Wistar or Fischer strain rats in a basal diet of known composition and the production of hyperplastic nodules and/or malignant hepatomas are noted. Relationship of nodular proliferation to tumorigenesis is studied by histological observations at the light and electron microscope levels.

Major Findings: This contract was moved during the past year from the University of Pittsburgh to Temple University, because of the move of the principal investigator. A great deal of time during the past year has been spent in getting new facilities at Fels Research Institute in order, in preparation for the studies supported by this contract. These facilities are now functioning smoothly. The contractor is now feeding animals regimens containing (a) 3'-methyl-dimethyl-aminoazobenzene (3'Me-DAB), (b) 3'-Me-DAB plus Cu (which prevents cancer induction), (c) aflatoxin B-1, (d) alpha-naphthyl-isothiocyanate, (e) lithocholic acid, (f) 2-AAF, and (g) ethionine. Regimens (a), (c), (f), and (g) induce liver cancer in rats while (b), (d), and (e) do not. Yet, all compounds induce severe and progressive chronic liver damage. Animals will be sacrificed at regular intervals of 1 to 2 weeks in order to compare very carefully the sequence of histologic and biochemical changes during carcinogenicity. Aflatoxin B-1, 2-AAF, and ethionine have been found to be particularly useful agents for induction of hyperplastic nodules.

Significance to Biomedical Research and the Program of the Institute: Hyperplastic nodules are a critical and potentially requisite, precancerous lesion which occurs prior to overt carcinomas. It is desirable to have as much biochemical and morphologic information as can be obtained on the events that characterize the early cellular response to carcinogens. These techniques may in the future become useful in screening procedures of suspected carcinogens.

Proposed Course: To continue to define morphological and biochemical correlations in hepatic hyperplastic nodules produced by carcinogens, with the aim of elucidating common cellular and molecular changes caused by these carcinogens.

Date Contract Initiated: January 20, 1964

Current Annual Level: $ 35,422

UNIVERSITY OF TENNESSEE (NIH-NCI-E-69-2077)

Title: Carcinogenic Studies of Polyurethanes

Contractor's Project Director: Dr. John Autian

Project Officer (NCI): Dr. Richard Bates

Objectives: Polyurethanes are widely used in the human environment and there is experimental data suggesting that some of these plastics may be carcinogenic. However, polyurethanes are made with widely diverse chemical structures and none of the studies indicating possible carcinogenicity has been done with materials of known chemical structure. Thus, this contract was established to test the variety of chemically different polyurethanes of known structure by intraperitoneal implantation into rats and mice to ascertain what role the chemical structure may play in the induction of tumors.

Major Findings: A total of 17 chemically different polyurethanes were implanted into groups of rats and mice. Mortality in mice was very high in the early phases of the study, but even in the animals which survived over one year no tumors were observed. In contrast, a high incidence of tumors were observed in the rats. Practically all of the tumors have been identified as fibrosarcomas. The incidence of tumors in their latent periods has varied considerably among the various polyurethanes.

Significance to Biomedical Research and the Program of the Institute: The wide scale use of polyurethanes for various types of products and building materials is raising questions as to the potential hazards of these materials when particulates or powders are inhaled or ingested by workers during their course of employment. Once absorbed by man these particles may degrade releasing carbamate or aromatic amine moieties or other chemical structures having possible carcinogenic activity. The data now available suggest that the tumors may have been due to (1) chemical carcinogenesis, (2) solid state carcinogenesis, or (3) a combination of both types of carcinogenesis. Those acting by a chemical mechanism are likely to pose a much more serious problem to man than those acting by a solid state mechanism. Thus, additional projects may be necessary to sort out which polyurethanes act by which mechanism.

Proposed Course: Information for the past year has led to the designing of new experiments in which an intensive study will be made on a polyurethane having a high potential for tumor formation and on a polyurethane having a low incidence of tumor induction. The objectives of these studies will be directed toward resolving whether solid state carcinogenesis or chemical carcinogenesis plays a predominant role in the induction of tumors in rodents. Additional experiments have been directed toward the importance of the site of implant in tumor production.

Date Contract Initiated: June 24, 1969

Current Annual Level: $ 68,379

SUMMARY REPORT

BIOLOGY AND IMMUNOLOGY SEGMENT

Fiscal Year 1972

The goals of the Biology and Immunology Segment are to apply knowledge in cell biology and immunology to the identification of carcinogenic agents and the prevention of cancers.

In Vitro Transformation Program

Three contracts dealing with definition of standard experimental conditions for in vitro carcinogenesis bioassay and for interaction between chemical and biocarcinogens have been initiated.

A contract with Meloy, Inc. has been in existence for approximately two years. The objective of this contract is to investigate the different cell lines as well as cell strains for their ability to respond to toxic and transforming ability of different known carcinogens. In addition methods to increase sensitivity to chemical carcinogens through the use of co-carcinogens and enzyme activators as well as other enhancing agents is under current consideration. Recently dose response relationships between members of a number of different classes of chemical carcinogens was established using cells derived from clones of Balb/3T3 cells. Transformed colonies when isolated and grown as cell lines exhibited properties of neoplasia not seen with control cells. Next in importance to have demonstrated that it is possible to transform 3T3 cells, is the modification of the assay so that transformed foci can be observed on a background of normal monolayer cells.

An eight month old contract is in existence with Biolabs, Inc. The objective of this contract has been to study the process whereby chemical carcinogens promote viral tumorigenesis and to determine if oncogenic or nonocogenic viruses may similarly promote tumorigenesis of known chemical carcinogens. Using an adenovirus SA-7, and primary hamster embryo cells it has been possible to show that pretreatment with carcinogens may increase transformation frequency. Concentrations of chemicals above those necessary to achieve maximal enhancement may reduce or completely inhibit viral transformation. These data suggest that the interaction between chemical carcinogens and certain host cell constituants facilitates the fixation of viral genomes responsible for transformation.

A new contract (two months old) with Ohio State is investigating dose-response of carcinogens on Syrian hamster and human embryonic lung lines in vitro which together with oncogenic and nononcogenic viruses and hormones may enhance in vitro transformation. The influence of gonadal and renal cortical hormones on in vitro carcinogenesis by chemicals and viruses alone or in combination may facilitate the transformation of human cells.

Immunology Program

A contract supported program based on studies of chemical carcinogens as allergens (haptens) presently consists of the following contracts:

1. Becton-Dickinson Research Center. Carcinogens as Allergens: Detection of Exposure to Carcinogens by Cell-Mediated Immunologic Reactions to the Carcinogens. Guinea pigs have been sensitized to several polycyclic hydrocarbons by several routes. The most important studies are those in which guinea pigs were sensitized by aerosol exposure to benzpyrene. Preliminary studies indicate that lymphocytes from these animals react in vitro to the carcinogen attached to an appropriate carrier. This is important because if reproducible these in vitro methods could make it possible to study human populations to determine which agents individuals have been exposed to and to what degree.

2. University of Texas, Medical School (San Antonio). Development of In Vitro Methods for the Detection of Cell-mediated Immunologic Reactivity to Chemical Carcinogens. This is a new contract. It is anticipated that reproducible methods will be developed for the detection of haptens (carcinogens) by in vitro analogues of delayed hypersensitivity.

3. Case Western Reserve. Specific Immunological Unresponsiveness to Chemical Carcinogens and its Influence on Tumorigenesis. This is a new contract. Animals will be rendered immunologically unresponsive to a given carcinogen and then tested for susceptibility to carcinogenesis by the agent. The purpose of this contract is to determine whether immune response to a carcinogen affects its capacity to produce cancer.

4. Brandeis University. Production and Detection of Antibodies to Chemical Carcinogens and other Small Molecules. This is a new contract. The immediate objective of this project is to obtain antibodies that react specifically with chemical carcinogens and other small molecules (haptens) of interest in cancer research. These antibodies will be used as reagents to detect the presence of corresponding haptens in the body fluids and tissues of animals and humans. In addtion, the haptens attached to appropriate macromolecular carriers will be used as antigens to detect the presence of antibodies to the haptens in the serum of animals and humans. These reagents will be tested for their suitability for the detection of exposure to environmental agents that may be related to cancer causation.

The following new contracts have been negotiated in this fiscal year:

1. Trudeau Institute, Saranac Lake, New York. The purposes of this contract are to develop a resource to provide mycobacteria for cancer research, to determine conditions for optimal preparation and storage of mycobacteria and to determine which mycobacteria are most effective in producing inhibition of tumor growth.

2. Department of Microbiology, University of Illinois, School of Medicine, Chicago, Illinois. The goal of this contract is to determine whether specific tumor immunity can be transferred in vivo with cell free extracts.

3. Childrens Hospital, District of Columbia. Detection of tumor specific cellular immune responses in children with tumors (in collaboration with Viral Oncology).

4. Department of Surgery, Georgetown University, District of Columbia. Development of in vitro tests for detection of tumor specific immune responses in humans with cancer (in collaboration with Viral Oncology).

Several contracts are directed toward developing better diagnostic tools for human carcinoembryonic antigens and to study the action of chemical carcinogens on complement components. A study of in vivo effects on complement of chemical carcinogens is now being concluded. The original observation that administration of nontoxic, carcinogenic doses of DEN to guinea pigs reduced serum C4 content to about 10% of control values has been confirmed. None of the other three carcinogens showed this effect. We are now investigating the in vitro effects of chemical carcinogens on the in vitro synthesis of C2 and C4 both in human and guinea pig tissue cells.

In a contract with Boston City Hospital we have initiated studies to devise a simple and rapid test for the presence of CEA in blood serum of humans. This test is not intended to replace the more refined and accurate radioimmuno assay for the antigen; its purpose is to permit large scale screening rapidly, cheaply and efficiently, for the presence or absence of a predetermined level of the antigen. Promising results show that a test that fulfills the above requirements can be devised that can detect nanogram/ml quantities of CEA in serum.

In a related contract, members of the Scripps Clinic are isolating CEA from human tissues with the goal of immunochemical characterization. That group will search for specific (non-embryonic) tumor antigens in these tissues.

CONTRACT NARRATIVES

AEC-NCI INTERAGENCY AGREEMENT (NCI-FS-(64)-13)

Title: NCI-AEC Carcinogenesis Program - Research on Co-Carcinogenesis

Contractor's Project Director: Dr. James Liverman

Project Officer (NCI): Dr. Allen Heim

Contract narrative is reported under the Summary Report for the Office of the Associate Scientific Director for Carcinogenesis.

BECTON, DICKINSON RESEARCH CENTER (NIH-NCI-E-71-2168)

Title: Carcinogens as Allergens: Detection of Exposure to Carcinogens by Cell-Mediated Immunologic Reactions to the Carcinogens

Contractor's Project Director: Dr. Jerry J. Tulis

Project Officer (NCI): Dr. Herbert J. Rapp

Objectives: To determine whether exposure of experimental animals to known carcinogens via various routes will result in the elaboration of in vivo (delayed hypersensitivity) and in vitro (lymphocyte transformation or macrophage inhibition) immunologic reactions. The ultimate goal of the program is the development of in vitro screening procedures to determine the potential hazards to man from exposure to environmental carcinogens.

Major Findings: Findings to date include the elaboration in experimental animals of immunologic sensitivity to several carcinogenic chemicals administered at low exposure doses and an apparent relationship between in vivo and in vitro immunologic reactions. Accomplishments include the development of a sophisticated aerosol apparatus that is used to expose animals to quantitated aerosols of carcinogens.

Significance to Biomedical Research and the Program of the Institute: The development of immunologic sensitivity in experimental animals upon exposure to known carcinogens as determined by in vivo and in vitro methods may provide insight to the environmental or occupational hazard and mechanism of action of carcinogens and provide information on environmental monitoring for these materials.

Proposed Course: Future work will include the screening of additional carcinogenic materials for elaboration of cell-mediated immunologic reactions, aerosol retention studies, and monitoring for humoral responses in animals exposed to carcinogens.

Date Contract Initiated: June 22, 1971

Current Annual Level: $106,579

BIOLABS, INC. (NIH-NCI-E-71-2164)

Title: In Vitro Study of Interaction Between Chemical and Viral Carcinogens

Contractor's Project Director: Dr. Bruce C. Casto

Project Officer (NCI): Dr. Joseph A. DiPaolo

Objectives: The specific objective of this contract is to study the process whereby chemical carcinogens promote viral tumorigenesis and to determine if oncogenic or non-oncogenic viruses may similarly promote tumorigenesis of

known chemical carcinogens. The system chosen by us utilizes cultures of primary hamster embryo cells and an adenovirus, SA7, known to be oncogenic for these cells. The major advantage of using this system is that rapid, reproducible and quantitative information can be obtained within a reasonably short time.

Major Findings: This contract has been in operation for eight months. Seventeen chemicals, including polycyclic hydrocarbons and alkylating agents known to induce transformation in vitro and/or malignancy in vivo, were diluted in acetone and added to primary hamster embryo cells at 18- or 2 hours prior to, or 3- to 4 hours after, the addition of an oncogenic adenovirus, SA7. An increased frequency of viral transformation of from 2- to 8-fold was observed when cells were treated with BP, 3-MCA, 7,12-DMBA, DBA (a,h or a,c), MAM acetate, or MNNG. BP, 3-MCA and 7,12-DMBA enhanced transformation by 2.5- to 3.5-fold when added 18 hours prior to virus, but depressed transformation by as much as 10-fold when added 4 hours after virus. Increases in transformation frequency were directly related to chemical concentration; however, in several experiments concentrations above those necessary to achieve maximal enhancement often reduced or completely inhibited viral transformation. These data suggest that the interaction between chemical carcinogens and certain host cell constituents facilitates the fixation of viral genomes responsible for transformation.

Significance to Biomedical Research and the Program of the Institute: The total program is directly related to the charge of the Etiology Area of the Institute. In view of the various hypotheses concerning chemicals, viruses, and cancer, and due to the inherent difficulties in studies in the intact animal, it is advantageous to study the various interactions in a controlled in vitro system. This would establish not only the influence of oncogenic and non-oncogenic viruses in promoting chemical carcinogenesis but the role of chemical carcinogens in promoting viral tumorigenesis.

Proposed Course: Work will continue on the nature of the interaction between adenoviruses, chemical carcinogens, and hamster cells, which leads to the enhancement of virus transformation. Chemical carcinogens, shown to enhance viral transformation, will be tested for their ability to enhance transformation of hamster cells by other chemical carcinogens. Evaluation of other virus and cell transformation systems will be conducted. An attempt will be made to develop a quantitative, routine focus assay for chemical transformation of hamster cells, that does not require the use of feeder cells. Transformed cells arising from the combined and independent action of virus and chemical will be characterized by: 1) growth rates, 2) cloning in soft agar, 3) tumorigenicity in hamsters, and 4) analysis of virus-specified antigens.

Date Contract Initiated: June 9, 1971

Current Annual Level: $64,917

CHILDREN'S HOSPITAL MEDICAL CENTER (NIH-NCI-E-71-2278)

Title: Effects of Carcinogens on In Vitro Synthesis of Complement Components

Contractor's Project Director: Dr. Harvey R. Colten

Project Officer (NCI): Dr. Tibor Borsos

Objectives: The main objectives of this contract are a study of the effects of selected chemical carcinogens and their non-carcinogenic analogues on the in vitro synthesis of complement components and a study of the ability of various metabolic inhibitors, hormones and other chemicals and biologically active molecules to counteract the effects of the carcinogens.

The contract is to be carried out in two phases. In phase I, the effects of at least ten carcinogens on the in vitro production of complement components are being studied. The carcinogens have been chosen from the following classes of chemicals: Aliphatic N compounds, polycyclic hydrocarbons, aromatic amines and heterocyclic compounds. The non-water soluble chemicals are introduced to the cultures in appropriate solvents, and the effects of the solvent on component synthesis is determined. For each tissue and chemical tested, toxicity levels are determined.

Toxicity is determined by comparing surviving cells in experiments and controls. Results considered positive if in the absence of toxicity, synthetic rates or levels are at least two-fold different in cultures treated with chemical carcinogens from those of controls.

In phase II, contractor shall study the effects of various agents for their ability to interfere with the effects of carcinogens on complement synthesis. These agents shall include hormones (such as cortisone), metabolic inhibitors (such as Actinomycin D) and other biologically active compounds selected by contractor in consultation with project officer. Appropriate controls to measure the effect of these agents alone shall be included.

Major Findings: The following chemicals have been tested so far:

Beta-Propriolactone
Coumarin
6-(p-Dimethylaminophenylazo) quinoline
N-N-Dimethyl-p-phenylazoaniline
3'-Methyl-4-dimethylaminoazobenzene
p-Aminoazobenzene
N-Nitrosodiethylamine
N-Nitrosodiphenylamine
N-Nitrosomethylurethan
Methylurethan
Ethyl Carbamate
Dimethylaminostilbene

Of the nitroso compounds, two were effective in inhibiting biosynthesis of the second (C2) and fourth (C4) components of complement by isolated guinea pig

peritoneal exudate (PE) cells. Nitrosomethylurethan, the more active of the two, was effective at a concentration of 1 x 10^{-4} M (the minimum dose that was tested). At this concentration, the agent led to more than a 90% inhibition of C2 and C4 biosynthesis with no apparent effect on cell morphology or on total cell survival during the observation period of 96 hours. Particularly at low concentrations of nitrosomethylurethan, the inhibition of C2 and C4 biosynthesis was readily reversible. This agent also inhibited total protein synthesis by PE cells. Preliminary experiments indicated that a concentration of nitrosomethylurethan ten times those used in this experiment had no effect on the stability or detection of biologically active C2 or C4. Interestingly, methylurethan, a non-carcinogenic analogue of nitrosomethylurethan, had no effect on C2 or C4 biosynthesis by PE cells even at a concentration of 1 x 10^{-3} M.

Nitrosodiphenylamine, an agent not thought to be carcinogenic guinea pigs, did inhibit C2 and C4 biosynthesis. At 10^{-3} M and 5 x 10^{-4} M, an 80 to 90% inhibition of C2 and C4 production was observed; 1 x 10^{-4} M nitrosodiphenylamine led to a 50% inhibition of C2 and C4 synthesis. Total protein synthesis by the PE cells was also inhibited by this agent. In addition, morphologic changes in the PE cells were noted in the presence of the nitrosodiphenylamine. These changes consisted of a rounding of the cells (less spreading) and an increase in cytoplasmic granularity. However, cell counts indicated no significant losses in total cell number in the presence of this agent over the 96-hour observation period. Based on those observations, it is likely that this agent has a generalized effect on PE cell function and that the inhibition of C2 and C4 biosynthesis is a reflection of this generalized effect.

Nitrosodiethylamine (DEN), a potent carcinogen *in vivo*, had no effect on C2 and C4 biosynthesis by PE cells, spleen, or liver fragments even at concentrations up to 1 x 10^{-3} M. Since two of the other nitroso compounds inhibit C2 and C4 biosynthesis *in vitro*, and DEN decreases C4 levels *in vivo*, it is possible that the DEN is converted *in vivo* but not *in vitro* to a compound that can affect macrophage function.

None of the azo dyes had any effect on C2 or C4 biosynthesis at doses up to 10^{-3} M.

Dimethylaminotranstilbene did not remain in solution except in the presence of at least 10% DMSO. This concentration of DMSO is toxic for the cells under the culture conditions. Therefore, an adequate evaluation of the effect of the carcinogen was not possible.

Significance to Biomedical Research and the Program of the Institute: The proposed contract would provide a relatively inexpensive, rapid way to examine the early events in carcinogenesis with particular emphasis on the effect of carcinogens on protein synthesis and specifically on the synthesis of immunologically active proteins. In addition, a sensitive bioassy for potential carcinogens might result from these studies. It should be noted that such work could lead, therefore, to both a fundamental and practical understanding of the mechanisms of chemical carcinogenesis.

Proposed Course: Under this contract we plan to complete the screening studies that were initiated in the first year of this contract and to explore particularly those areas that appear fruitful on the basis of these early results. Specifically, only one aromatic amine has been examined thus far for its ability to affect in vitro complement biosynthesis; eight other aromatic amines and related compounds will now be studied. In addition, it would be valuable to examine the effect on complement biosynthesis of four other compounds, two of them hydrocarbons and two azoxy compounds. Accordingly, the plan is to study the effect of these 12 compounds on C2 and C4 biosynthesis in vitro.

Date Contract Initiated: June 1, 1971

Current Annual Level: $37,380

CORDIS CORPORATION (NIH-NCI-E-69-2076)

Title: Effects of Carcinogens on Complement Non-specific Immune System

Contractor's Project Director: Dr. Seymour P. Halbert

Project Officer (NCI): Dr. Tibor Borsos

Objectives: There is evidence that carcinogens interfere with the body's immune defenses. It is not known, however, whether carcinogens have any effect on complement components in man or experimental animals.

An objective of this study is to determine whether carcinogens are capable of inhibiting the in vivo action of complement components and if so, whether the process of inhibition could be useful in identifying and characterizing carcinogens. Groups of guinea pigs (Hartley strain) were given intraperitoneal injections of known potent carcinogens, of differing chemical class. Serum samples were obtained after 0, 2, 14 and 28 days. These were assayed for hemolytic activity of total complement and for all of its components, as well as for the inhibitors of C1, C3 and C6. The animals were examined for malignancies at appropriate times and additional serum samples obtained if tumors were present.

Major Findings: Two carcinogens, N-nitrosodiethylamine (NDEA) and N-hydroxy-2-acetylaminofluorene (N-OH-AAF) and their corresponding non-carcinogenic analogues, N-nitrosodiphenyl-amine (NDPA) and antylaminofluorene (AAF) have been studied for their effects on guinea pigs. Single intraperitoneal injections of varying amounts were given to determine toxicity and effects on complement component levels. Guinea pigs given as much as 18 mg per 100 gram body weight of N-OH-AAF or AAF survived the injections. On the other hand, deaths occurred with animals given 5 mg to 45 mg/100 gram body weight of NDEA or NDPA. In all cases, no significant or consistent changes in complement component levels were noted.

The chemicals were studied for effects on guinea pigs following prolonged administration. NDEA at doses that led to death of the study group within 9 weeks gave rise to a decrease in complement component levels, particularly C4, and including C3, C1, C2, C6, C8 and C9. No liver pathology was noted from gross examination of the organ. In doses half the amount given in the above group, NDEA gave rise to definite liver growth with metastatic lesions in the lungs. In all animals, C4 levels were decreased as early as the second week and persisted until the death of the animal. No other component level changes were noted. With N-OH-AAF or AAF, no gross lesions were noted in the three animals that have died so far. No complement component changes were observed in all animals.

Significance to Biomedical Research and the Program of the Institute: This contract explores the in vivo effects of various carcinogenic chemicals on the complement system. Complement is an important part of the immune defense mechanism. It is thought that immune defense mechanisms have an important role in surveillance of tumor cells. Interference with the operation of the immune mechanism may lead to impairment of surveillance and the appearance of cancer.

Proposed Course: Contract terminated as of June 20, 1972.

Date Contract Initiated: June 20, 1969

Current Annual Level: $97,733

MALLORY INSTITUTE OF PATHOLOGY FOUNDATION (NIH-NCI-E-71-2276)

Title: Detection of Carcinoembryonic Antigens in Humans

Contractor's Project Director: Dr. Norman Zamcheck

Project Officer (NCI): Dr. Tibor Borsos

Objectives: This project is designed to develop reagents and methods that are suited for the detection of carcinoembryonic antigens (CEA) in serum. It is envisioned that the new test that will be developed in this project will have the following properties:

1) The test can be performed on serum samples of 0.5 ml or less.
2) The test is specific for CEA, i.e., no other serum constitutent will interfere with the specific reaction.
3) The test is capable of detection of between 10 and 100 ng of CEA per ml of sample.
4) The test can be performed in less than 6 hours excluding preparation of reagents.
5) The test can be performed by technical personnel in a clinical diagnostic type laboratory.

Major Findings: Monospecific rabbit antisera have been produced to CEA. These antisera are capable of reacting with as little as 1 ng of CDA and their activity at this level of sensitivity can be detected by counter electrophoresis.

In order to test further the specificity of the rabbit antiserum, we isolated the gamma globulin antibodies from the goat and rabbit anti-CEA antisera and tested them by immunoelectrophoresis for antibodies to (a) normal human serum, (b) CEA from Boston, Nutley, and Montreal, and (c) PCA extracts of cirrhotic liver. These tests yielded multiple precipitin lines with the CEA antigens indicating that the CEA's from Boston, Montreal, and Nutley were either not homogenous or else contained "fragments" of CEA. In addition, the rabbit anti-CEA antiserum did not react with normal human serum or with PCA extract of cirrhotic liver, thus differing from the goat anti-CEA antisera.

These experiments were repeated using a many-fold concentrate of rabbit anti-CEA antiserum in order to eliminate the possibility that the non-reactivity between the rabbit anti-CEA antiserum and the extract of cirrhotic liver was due to an initial antibody concentration too low to form an immuno-precipitin reaction. These experiments confirmed the above studies by showing non-reaction of anti-CEA antiserum with PCA extracts of cirrhotic liver.

Significance to Biomedical Research and the Program of the Institute: We are interested in developing screening techniques that permit the identification of populations at risk to cancer. Recent evidence indicates that colon cancer in man is accompanied by the appearance in serum of a carcino-embryonic antigen. The identification of an antigen characteristic of colon cancer will help elucidate the etiology of colon cancer and will yield information regarding control of this form of cancer.

Proposed Course: The rapid screening method will be further developed, whereby its specificity, sensitivity and usefulness will be compared to the standard radio-immuno assay for CEA. Collection of sera from patients with varying neoplastic and non-neoplastic sera will continue under the supervision of the pathologist who has been added to the contract for this purpose.

Date Contract Initiated: June 1, 1971

Current Annual Level: $78,028

MELOY LABORATORIES, INCORPORATED (NIH-NCI-E-70-2201)

Title: Definition of Standard Experimental Conditions for a Reproducible *In Vitro* Carcinogenesis Bioassay

Contractor's Project Director: Dr. John E. Verna

Project Officer (NCI): Dr. Joseph A. DiPaolo

Objectives: 1) An investigation of different cell lines as well as cell strains for their ability to respond to the toxic and transforming ability of various levels of different known carcinogens. 2) The use of known co-carcinogens and enzyme activators as well as enhancing agents will be undertaken to increase sensitivity of the current testing procedures. 3) Increased sensitivity to chemical carcinogens will also be considered following halogenated and unhalogenated DNA precursors as well as following sequential exposure to physical agents. 4) The reliability of any and all in vitro bioassays will be confirmed through the use of double-blind testing methods and evidence that the transformation results in the formation of tumors when the cells are inoculated into the appropriate hosts.

Major Findings: During the past contract year, dose-response relationships between members of a number of different classes of chemical carcinogens were established using cells derived from clones of Balb/3T3 cells. With the exception of the nitroso compounds, carcinogens produced transformations which when isolated and grown as cell lines exhibited properties of neoplasia not seen with control cells. The isolation of normal colonies from control dishes and nontransformed colonies from carcinogen treated cultures failed to produce tumors. Next in importance to having demonstrated that it is possible to transform and establish tumor producing cell lines is the possibility that instead of examining dishes and bottles for transformed colonies, it may be possible to determine transformed foci on the background of a normal monolayer. Experiments for the direct involvement of oncogenic virus in induction of chemical transformation have been negative. The cloned lines of transformed cells were tested for murine leukemia antigen and DNA polymerase activity.

Significance to Biomedical Research and the Program of the Institute: The eventual goal of this program is to utilize the mammalian cells in tissue culture for the development of in vitro models for screening environmental factors that are carcinogenic to humans. This research has a direct relevance to etiology of cancer. The development of a series of in vitro assays should enable cancer investigators to rapidly determine the carcinogenicity of a wide variety of chemicals as well as to carry out fundamental studies related to the process of carcinogenesis so that prophylactic measures may be taken to prevent cancer. In this fashion an entirely new approach becomes available for the study of chemical carcinogenesis.

Proposed Course: The proposed work is to further develop sensitive, reproducible in vitro bioassays for the detection of chemical carcinogens. A major goal is a simple, rapid, easily scored system using mammalian cells. Conditions are standardized to minimize any external fluctuations. This is required to determine the fundamental criteria that distinguish the toxic and transforming potentialities of various carcinogenic compounds. Because of the diverse nature of the compounds to be examined, a large number of experimental variables such as the time of exposure, number and types of cells, and sequence of exposure as well as conditions of treatment of cells must be examined. If the transformation is due to a direct inductive activity, a chemical carcinogen would be expected to produce a variety of new antigens in the cells they have transformed. Therefore, some funds are required for supervision of immunological experiments as well as for animals.

Date Contract Initiated: June 26, 1970

Current Annual Level: $167,300

UNIVERISTY OF NEBRASKA MEDICAL CENTER (NIH-NCI-E-70-2203)

Title: Development of an In Vitro Test for Chemical Carcinogenesis

Contractor's Project Directors: Dr. Melvin Greenblatt and Dr. Giamperio di Mayorca

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The project was to develop experimental conditions for a sensitive and reproducible in vitro bioassay for the determination of the carcinogenic potential of chemical compounds. The following approach was used:

1) An easily cultured stable diploid cell line was used to avoid the possibility of dealing with cancerous cells prior to treatment.

2) Cells were exposed to the chemicals tested briefly to avoid long-term selection effects and after exposure, were cloned for quantitation of transformation.

3) Some chemicals are known to undergo metabolization in vivo, leading to production of active carcinogens. These chemicals may be inactive in an in vitro bioassay; to overcome this difficulty, the detector diploid cells were exposed, in implanted semi-permeable diffusion chambers, to the body fluids of hamsters treated with the chemicals under test. After exposure, the cells will be cloned.

4) Carcinogenic activity in animals was correlated with the transformation of cells in vitro.

5) Attempts were made to standardize in vitro bioassay conditions as to be reproducible under routine laboratory conditions.

Major Findings: We have succeeded in defining conditions for and developing and in vitro system in which substances may be tested for their carcinogenic potential. Essentially, BHK_{21} cells are briefly (one hour) exposed to the potential carcinogen and the surviving cells are subsequently tested for transformation as determined by their ability to grow in soft agar.

Conditions were defined by using two known carcinogens; dimethylnitrosamine (DMN) which requires metabolic activation for its tumor producing effect and nitrosomethyl-urea (NMU), a potent mutagen. Toxic effects of both compounds were studied; relatively high concentrations of DMN (up to 25 mg/ml) did not affect the plating efficiency of BHK, whereas as little as 0.001 mg/ml of NMU resulted in 10% survival of the cells. Preincubation of DMN with liver homogenate showed no significant increase in toxicity. The surviving cells were then either put in agar directly to measure transformation frequency

(DMN - 6×10^{-6}) or first cloned and the clones showing a transformed morphology regrown and then plated in agar (DMN, NMU 10^{-4}). In both cases, transformed clones were obtained. It must be emphasized that only a small fraction of the clones showing a transformed colonial morphology showed the transformed phenotype by plating in agar. Morphological transformation alone, therefore, cannot be used as a criterion to evaluate a substance's carcinogenic potential. In fact, clones which showed a transformed morphology, but did not plate in agar, did not cause tumor production in animals.

Sequestering the cells in intraperitoneal diffusion chambers and administering the test compound to the animal resulted in good survival of the cells. Studies on the transformation of these cells are pending investigation.

Eighteen clones were tested for oncogenicity in hamsters by inoculating various cell concentrations derived from 10 clones transformed by DMN; 5 by NMU, 2 viral transformed clones and untreated BHK. Excellent correlation was obtained between ability of plating in soft agar assay and tumor production in vivo involving more than 200 test animals. At high concentrations of cells (10^6 - 10^7/animal) visible nodules were found after one week. Tumors appeared after three weeks when as little as 500 cells were inoculated Histopathological studies showed highly anaplastic neoplasms which although were grossly circumscribed also presented metastases in the lymph nodes, lungs and other organs.

All of our chemically transformed clones and a single spontaneously transformed clone showed a temperature dependence for clonal morphology and the ability to grow in soft agar. Polyoma transformed cells did not show this phenomenon. By analogy with bacterial and viral ts mutants, this phenomenon suggests an alteration in a single genetic locus of the cell, probably a single base-pair.

Significance to Biomedical Research and the Program of the Institute: This project will contribute to the priority of devising much needed rapid and inexpensive bioassay methods for testing and screening chemicals of potential carcinogenic nature.

Comparative studies of chemical transformation in the intact animal bearing diffusion chambers with in vitro systems should be pursued. It would be of value to determine if the transformation rate is increased using the in vivo system.

Because of the potential usefulness of cells that are temperature sensitive for transformation in exploring the mechanisms for chemical carcinogenesis, it would be important to learn how general this phenomenon is by studying cell clones produced by exposure to other chemicals, cell clones derived from spontaneous tumors, expand studies with viral transformed cells to other viruses, etc. If the phenomenon is general, it may provide a method for distinguishing between spontaneous or chemically induced transformed cell lines and viral transformed lines. It may be of use in distinguishing spontaneously or chemically transformed tumors from viral induced tumors in animals or humans and help to elucidate the underlying mechanism by which a cell becomes transformed.

Proposed Course: To continue as outlined above.

Date Contract Initiated: June 26, 1970

Current Annual Level: $194,098

UNIVERISTY OF UTAH (NIH-NCI-E-71-2272)

Title: Carcinogenesis Bioassay Resource for Determining the Effect of Chronic Immunosuppression on Physical and Chemical Carcinogenesis.

Contractor's Project Director: Dr. Ernst J. Eichwald

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: It has become evident that multiple factors, such as nutrition, defense mechanisms of the host, and age, affect the carcinogenic process of environmental carcinogens. The effect of immunosuppression on physical and chemical carcinogenesis is one such factor. This proposed study would complement a study of the carcinogenicity of immunosuppressive agents, which is in progress under a different contract. Five immunosuppressive agents in common clinical use (prednisone, cyclophosphamide, anti-lymphocyte globulin, azathioprine, and methotrexate) would be utilized in this study. Since most chemical carcinogens are immunosuppressive agents, an attempt to understand the role of immunosuppression in carcinogenesis will be made.

Major Findings: Dose-response studies are in progress to determine threshold and suboptimal carcinogenic regimens: 1) skin carcinogenesis by chronic exposure to benzo(a)pyrene or ultraviolet irradiation in mice; and 2) subcutaneous carcinogenesis by a single injection of methylcholanthrene in rats. Meticulous measurement of the doses of ultraviolet irradiation and polynuclear hydrocarbons are being made. The establishment of effective, nontoxic and chronic immunosuppressive regimens will continue into the second contract year due to the low therapeutic index of some of the chemical immunosuppressive agents, especially Cytoxan. Techniques for the production in rabbits of liter amounts of ALG have been developed. A lymphocytic cell membrane preparation was more effective than while lymphocytic cells in the production of ALG.

Significance to Biomedical Research and the Program of the Institute: As shown by clinical and experimental investigations, cellular immunity may be an important arm of host defense and a modifier of the carcinogenic process. Interestingly, chemical carcinogens commonly are immunosuppressive agents. This project will explore the effect of chronic immunosuppression on chemical and ultraviolet carcinogenesis. In addition, efficacious immunosuppressive regimens will be established in two animal species, i.e. rat, and mouse. This information can then be used in other animal models of carcinogenesis presently under study in the contract program.

Proposed Course: Phase I studies will continue into the first half of the second contract year. It will be determined whether or not chronic immunosuppression will affect the latency, incidence, histological type and development of metastases of tumors induced by threshold and suboptimal carcinogenic levels of ultraviolet light or polynuclear hydrocarbons administered by cutaneous routes. The degree of immunosuppression will be monitored by skin grafting an assay of cellular immunity. A total of 4,000 inbred animals will be used in this study.

Date Contract Initiated: May 7, 1971

Current Annual Level: $76,770

SUMMARY REPORT

CHEMISTRY AND MOLECULAR CARCINOGENESIS SEGMENT

Fiscal Year 1972

Organization and orientation of intramural and extramural scientists for the purpose of handling programmed research by the medium of contracts has been undertaken by this newly established Segment this year. The NCI staff on our Segment are Drs. Bader, Dingman, Gelboin, Green, Hatfield, Kakefuda, Ness and Peacock. This group has met on many occasions for the purpose of selecting outside consultants, reviewing of ongoing contracts, evaluation of unsolicited proposals, and discussion of the projects initiated by members of the group.

A group of outstanding scientists of varied interests, all of whom have accepted our invitation to serve as advisors, has been selected to form the Segment Advisory Group. This group will work with the NCI staff to devise a coordinated program in the difficult and complex field of chemical and molecular control of carcinogenesis.

A separate group within our Segment, under the leadership of Dr. John Bader, has been formed to develop a coordinated study into the genetics of somatic vertebrate cells, with the express aim of developing resources for the collection and distribution of well-characterized mammalian cell mutants. Dr. Bader is assisted by an ad hoc advisory group consisting of Dr. Jerard Hurwitz, Dr. Harvey Ozer, Dr. Theodore Puck and Dr. Louis Siminovitch.

The major interest in our contract activities is the study of carcinogenesis as a process, and the development of concepts and tools which may be necessary for this goal. The contracts now in force, or planned to be in operation, cover a limited portion of this large and complex area. The segment supports studies on polynuclear hydrocarbons, their metabolic conversion to carcinogens, the chemical reactivity of these and similar compounds, and the biological effect of such agents on cells in tissue culture. Genetic factors are defined in human populations that seem favorable to the development of the carcinogenic process in cancer of the pancreas, and attempting to gain insight into the genetic control of factors affecting carcinogenesis in cell cultures. We are planning studies on regulatory mechanisms in vertebrate tissue which may influence the development of malignancy, especially with respect to prolactin and its effect on the carcinogenic process in mammary tissue, and a general study of proteins binding to the nucleus which may be of significance in the control of carcinogenesis in tissues of all types. We are proposing a contract for the development of specialized media for electrophoretic analysis to facilitate the study of macromolecules and cellular organelles responsible for cellular regulation processes.

Our major goal for the next year is the development of a rational, imaginative, and executable plan to provide significant increase in our knowledge of the process of carcinogenesis and to reconsider which of our programs should be replaced in order to achieve this end.

The major accomplishments of the contracts in the Chemistry and Molecular Carcinogenesis Segment in fiscal year 1972 are as follows:

At the Home for the Jewish Aged (NIH-NCI-E-71-2269), the haptoglobin types in serum of 44 patients with carcinoma of the pancreas have been shown to be significantly different from those of patients with conditions clinically like carcinoma of the pancreas and from the normal resident population of the Philadelphia Geriatric Center.

At New York University (NIH-NCI-E-71-2183), several mutant lines from mouse (3T3) and hamster (BHK) cells have been isolated and partially characterized. All mutants tested thus far by complementation analysis are recessive, and one mutant seems to be specifically defective in DNA synthesis.

At Johns Hopkins University (NIH-NCI-E-71-2169), a cell-free microsomal system has been established which incorporates precursor molecules into heme. This is the first essential step of this program.

At the Weizmann Institute of Science (NIH-NCI-E-70-2217), reversion of the transformed state of cells treated with dimethylnitrosamine to the loss of malignancy has been observed. Certain chromosomal groups seem to be identified with expression and suppression of malignancy by polyoma virus. Thus, the data suggest that viral and nonviral carcinogens induce malignancy by inducing similar chromosomal rearrangements. Recent research indicates that different degrees of stability of the malignant state appear related to differences in the balance of chromosomally located factors that determine the expression and suppression and transformed properties.

CONTRACT NARRATIVES

AEC-NCI INTERAGENCY AGREEMENT, OAK RIDGE NATIONAL LABORATORY (NCI-FS-64-13)

Title: NCI-AEC Carcinogenesis Program - Research on Co-Carcinogenesis

Contractor's Project Director: Dr. James Liverman

Project Officer (NCI): Dr. Allen Heim

Contract narrative is reported under the Summary Report for the Office of the Associate Scientific Director for Carcinogenesis.

AEC-NCI INTERAGENCY AGREEMENT, UNIVERSITY OF CALIFORNIA (NCI-FS-71-58)

Title: Studies on Polycyclic Hydrocarbon Activation, and Interaction with Oncogenic Viruses and Cell Macromolecules

Contractor's Project Director: Dr. Melvin Calvin

Project Officers (NCI): Dr. Harry V. Gelboin
Dr. Timothy O'Connor

Objectives: To define the chemically reactive position of polycyclic hydrocarbons and to determine the interaction of the reactive intermediate with cell macromolecules and the nature of interaction with oncogenic viruses.

Major Findings: This project just got started this year and preliminary experiments have been done in terms of setting up the methodology and the laboratory facility. These have been successfully accomplished but no substantive results have been reported. Progress has been made on using the pyridine-N-oxide method of oxygenation of hydrocarbons and some preliminary results indicate that mammary tumors induced by DMBA can be inhibited by rifampicin, an inhibitor of reverse transcriptase.

Significance to Biomedical Research and the Program of the Institute: One of the most important aspects of carcinogenesis in humans may be related to polycyclic hydrocarbons which are a principal component of tobacco smoke and air pollution. Understanding the nature of activation of hydrocarbons, the chemical reactivity of each site, the role of enzymes in the activation and binding of the reactive form of the carcinogen to macromolecules of the cell and to components of oncogenic viruses may clarify the role and effect of enzymatic activation of polycyclic hydrocarbon carcinogenesis and the role of these agents in the activation of tumor viruses.

Proposed Course: To perform the studies outlined in the first year's proposal on the above problem.

Date Contract Initiated: April 12, 1971

Current Annual Level: $ 150,000

HOME FOR THE JEWISH AGED (NIH-NCI-E-71-2269)

Title: Study of Serum Haptoglobin Types in Patients with Carcinoma of the Pancreas

Contractor's Project Director: Dr. Henry Altschuler

Project Officer (NCI): Dr. Andrew Peacock

Objectives: This is one of two identical projects, the objective of which is to identify persons who, because of their genetic constitution, may stand an increased risk of malignant disease of the pancreas. Serum samples are collected from individuals with diagnosis of cancer of the pancreas and other individuals, both healthy and with various diseases, to form a control group for the pancreatic cancer group. The serum haptoglobin type is determined for each serum and a determination made whether the group of pancreatic cancers differ from the control groups with respect to the distribution of haptoglobin gene. Subtype determinations will be performed on a randomly selected

subsample of the patients with carcinoma of the pancreas and the normal control group.

Major Findings: Diagnosis of 44 patients with carcinoma of the pancreas has been confirmed and haptoglobin type determined. The percentage distribution in each phenotype was as follows:

Phenotype	Percentage
1-1	22.8
2-1	31.8
2-2	45.4

Twenty six patients who had conditions clinically like carcinoma of the pancreas were studied with the following percentage distribution:

1-1	7.7
2-1	69.2
2-2	45.4

Two hundred and one aged individuals, members of the resident population of the Philadelphia Geriatric Center, were typed as a possible control population. Results were as follows:

1-1	7.0
2-1	50.7
2-2	42.3

A statistically significant difference exists between the patients with carcinoma of the pancreas and the resident population.

Significance to Biomedical Research and the Program of the Institute: This project falls under the general heading "Identification of Susceptible Persons", which is an established goal of the total program. This project attempts to determine whether genetic characteristics believed to have significance with respect to leukemia and probably also breast and lung cancer are significant in cancer of the pancreas. The need to define differences between those persons who are susceptible to some forms of malignant disease and those who are not is well recognized; this project may be helpful in this regard in establishing a possible marker characteristic for a very frequent form of cancer in man for which very few etiological clues presently exist.

Proposed Course: Approximately 50 samples of serum from patients with cancer of the pancreas may be expected during the coming year. Data will be derived to confirm and expand upon the interesting results derived to date. The racial and age distribution in the control group will be defined based upon the characteristics found for the cancer group. An attempt will be made to form additional controls, including older persons (65-90 years) of various ethnic strains (i.e. from denominational old age homes) and from persons hospitalized for causes other than pancreatic cancer. Approximately 500 such serums will be studied.

Date Contract Initiated: April 30, 1971

Current Annual Level: $ 35,274

JOHNS HOPKINS UNIVERSITY (NIH-NCI-E-71-2169)

Title: Studies on the Regulation of the Heme Moiety of P-450 in Relationship to the Carcinogen Metabolizing Activity

Contractor's Project Director: Dr. Ephraim Y. Levin

Project Officer (NCI): Dr. Harry V. Gelboin

Objectives: To determine the mechanism of regulation of heme synthesis and degradation and to examine this regulation in respect to carcinogen metabolism.

Major Findings: This project was started relatively late in the year. Significant progress has been made in this short time. A cell free microsomal system has been established which can incorporate precursor into heme. This suggests that this project has good potential for success.

Significance to Biomedical Research and the Program of the Institute: Understanding the regulation of each component of the carcinogen metabolizing enzyme system relates to the understanding of the carcinogenic process.

Proposed Course: To continue to study the regulation of P-450 heme moiety.

Date Contract Initiated: June 25, 1971

Current Annual Level: $ 42,758

NEW YORK UNIVERSITY (NIH-NCI-E-71-2020)

Title: Alkylating Agents as Carcinogens and Anti-Carcinogens

Contractor's Project Director: Dr. Benjamin L. Van Duuren

Project Officer (NCI): Dr. Harry V. Gelboin

Objectives: Study of alkylating agents as carcinogens and anticarcinogenic agents in cancer induction.

Major Findings: Eighteen compounds of the alkylating type are currently on test. These include chemicals of industrial use as well as several anesthetics which are currently under test. These are being tested in 1400 mice.

Significance to Biomedical Research and the Program of the Institute: The future accomplishments of this group are expected to be in the realm of identifying the carcinogenic hazard of industrial chemicals. The identification and removal of these chemicals from the industrial environment is in the interest of the Government and cancer research.

Proposed Course: To continue to examine epoxide and alkylating agents particularly those of industrial importance to the carcinogenic process.

Date Contract Initiated: October 1, 1970

Current Annual Level:$ 105,681

NEW YORK UNIVERSITY (NIH-NCI-E-71-2183)

Title: The Isolation, Propagation, and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions

Contractor's Project Director: Dr. Claudio Basilico

Project Officer (NCI): Dr. John P. Bader

Objectives: The objectives of the project are the induction, isolation, propagation, storage, and distribution of mutant vertebrate cells. The cells will be selected for mutations in specific biochemical areas and characterized with respect to the biochemical lesion. Specifically, temperature sensitive mutants of mouse embryo (BALB/C 3T3) cells, and possibly hamster (BHK) cells, will be isolated after exposure to mutagenic chemicals. Selection and isolation will proceed as outlined in the proposal. Initial biochemical selection should be for defects in DNA or RNA synthesis. At the request of the project officer, isolated mutant cells will be made available for distribution at any stage in their characterization, along with the most current information on the characterization.

Major Findings: Several mutant isolates of mouse (3T3 BALB/C) and hamster (BHK) cells have been propagated which multiply faster at 33 degrees than at 39 degrees C. Thus far only the hamster cells have been even partially characterized and these show the following characteristics: (a) the efficiency of plating (ability of single cells to grow) at 39 degrees is less than 0.0001 of that at 33 degrees, (b) reversion frequency is less than 0.00001, but can be increased by re-exposure of the mutant cells to a mutagen, (c) one mutant stops growing within 4-6 hours after shifting to the higher temperature; other mutants continue to divide for approximately one generation (12 hours), (d) all the mutants tested thus far by complementation analysis are recessive, and (e) one mutant seems to be specifically defective in DNA synthesis; in other the biochemical lesion has not been identified.

Significance to Biomedical Research and the Program of the Institute: Studies in the induction of cancer by chemical and physical agents, as well as biological agents, are currently limited by the cells available for such studies.

The role of DNA synthesis or RNA synthesis, or specific enzymes in carcinogenic processes may be elucidated through the use of cells with mutations in specific metabolic pathways. For example, the effects of a chemical carcinogen on cells in which DNA synthesis is suppressed cannot now be examined, since all effective chemical inhibitors of DNA synthesis are toxic; a mutant conditionally defective in DNA polymerase, or cytidylate reductase, or thymidine synthetase, could be used to examine this problem. Initially, the project was restricted to common metabolic pathways, since these would be most generally useful, but eventually mutants in specialized pathways could be derived to examine such things as enzymes required for conversion of potential chemical carcinogens to the active form, or the role of specific cell membrane components in carcinogenesis.

The prevention or cure of cancer may and probably will require an understanding of the process or processes which convert a normal cell to a tumorigenic one. The value of genetic manipulation in understanding regulatory processes has clearly been demonstrated in microbial systems during the past two decades. The isolation of specific mutated metazoan cells is now technically feasible, and we can anticipate their value in studies on the carcinogenic process. It is likely that essential information necessary to the prevention or cure of certain cancers can only be derived through the manipulation of such cellular mutants.

Proposed Course: It is estimated that this project will require three years for completion.

Date Contract Initiated: June 2, 1971

Current Annual Level: $ 85,555

UNIVERSITY OF TEXAS (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE)
(NIH-NCI-E-71-2268)

Title: Study of Serum Haptoglobin Types in Patients with Carcinoma of the Pancreas

Contractor's Project Director: Dr. David Anderson

Project Officer (NCI): Dr. Andrew Peacock

Objectives: This is one of two projects the objective of which is to identify persons who, because of their genetic constitution, may stand an increased risk of malignant disease of the pancreas. Serum samples are collected from individuals with diagnosis of cancer of the pancreas and other individuals, both healthy and with various diseases, to form a control group for the pancreatic cancer group. The serum haptoglobin type is determined for each serum and a determination made whether the group of pancreatic cancers differs from the control groups with respect to the distribution of haptoglobin gene.

Major Findings: The contractor has found only 18 patients with carcinoma of the pancreas. Fifteen of these yielded serums suitable for haptoglobin typing. The numbers are too few to be significant. The project director has revised his collection mechanism to insure a larger number of carcinoma patients in the second year.

Significance to Biomedical Research and the Program of the Institute: This project falls under the general heading "Identification of Susceptible Persons" which is an established goal of the total program. The project attempts to determine whether genetic characteristics believed to have significance with respect to leukemia and probably also breast and lung cancer are significant in cancer of the pancreas. The need to define differences between those persons who are susceptible to some forms of malignant disease and those persons who are not is well recognized; this project may be helpful in this regard in establishing a possible marker characteristic for a very frequent form of cancer in man for which very few etiological clues presently exist.

Proposed Course: Approximately 50 samples of serum from patients with cancer of the pancreas may be expected during the coming year. Data from previous studies (on breast and leukemia) suggest that about 100 samples may be required to show the expected differences. The racial and age distribution in the control group will depend on the characteristics found for the cancer group. An attempt will be made to form additional controls, including older persons (65-90 years) of various ethnic strains (i.e., from denominational old age homes) and from persons hospitalized for causes other than pancreatic cancer. Approximately 500 such serums will be studied.

Date Contract Initiated: May 19, 1971

Current Annual Level: approx. $ 25,000

THE WEIZMANN INSTITUTE OF SCIENCE (NIH-NCI-E-70-2217)

Title: The Role of the Enzyme Aryl Hydrocarbon Hydroxylase and its Induction in Polycyclic Hydrocarbon Carcinogenesis

Contractor's Project Director: Dr. Leo Sachs

Project Officer (NCI): Dr. Harry V. Gelboin

Objectives: To study the effect of polycyclic hydrocarbon activated metabolites on cytotoxicity, mutagenicity, and transformation of human cell cultures. To study the chromosomal control of chemical carcinogens with refined methods of chromosome identification and to particularly study the chromosomal control of the microsomal carcinogen metabolizing enzyme system. This will be done in normal, transformed and revertant cells.

Major Findings: Cells can be transformed by the chemical carcinogen dimethylnitrosamine which will revert under certain circumstances to relatively

normal states or at least to a loss of malignancy. A chromosomal balance at these various states has been examined and found that certain chromosomal groups seem to be identified with the expression and suppression of malignancy by cells transformed by polyoma virus. Thus, the data suggest that viral and nonviral carcinogens induce malignancy by inducing similar chromosomal rearrangements. In other aspects of the study the stability of the reverted state has been analyzed and the data has been obtained on the incidence of reversion as well as re-reversion to the malignant state. The most recent aspects have shown that when cells revert to the malignant state they contain a high frequency of segregance which lose this property rapidly. The different degrees of stability appear related to differences in the balance of chromosomally located factors that determine the expression and suppression and transformed properties.

Significance to Biomedical Research and the Program of the Institute: Polycyclic hydrocarbons represent major contaminants in the environment and are serious threats to the human organism in a highly industrialized society. The enzyme system dealing with these agents is aryl hydrocarbon hydroxylase, and our findings indicate that the enzyme system is responsible for the activation of the hydrocarbon to a toxic and probably a carcinogenic form. The observation that transformed cells can revert back to normal properties is an exciting and important finding that may enable an understanding of conditions favoring a reversion of carcinogen transformed cells back to a normal state.

Proposed Course: To further clarify the relationship between the presence of the enzyme, the metabolism of carcinogenic hydrocarbons, and malignant cell transformation. Inhibitors of the enzyme, such as 7,8-benzoflavone, will be tested for their effect on cell transformation induced by carcinogenic hydrocarbons other than benzo(a)pyrene. The revertants produced from transformed cells without detectable enzymes will be tested for their enzyme activity in order to determine whether the suppression of malignancy results in the re-appearance of the enzyme in the reverted cells. Revertants from cells transformed by carcinogenic hydrocarbons and other chemical carcinogens will also be tested for their susceptibility to the cytotoxic and cell transforming activity of carcinogenic hydrocarbons. This contract has now added an investigator who has had extensive experience in the metabolism of polycyclic hydrocarbons in cell culture. He will look at the profile of metabolites under different conditions of enzyme level and in the normal, malignant, reverted and re-reverted state.

Date Contract Initiated: May 26, 1970

Current Annual Level: $ 75,400

SUMMARY REPORT

COLON CANCER SEGMENT

Fiscal Year 1972

The goal of the contract program of the Colon Cancer Segment is to identify the environmental carcinogen(s) responsible for colon cancer in the U.S. environment. Its methods are to supplement the Demography Area's studies on Japanese (who show a sharp increase in incidence upon migration from Japan to the United States), and to develop other laboratory and epidemiologic studies that will amalgamate the migrant program and the several relevant Carcinogenesis Area investigative programs into a coordinated whole. The general logic of program areas has been formalized into a linear array. As we assemble a group of advisors and familiarize them with the program, we expect to develop the plans further with more complete specification of individual projects. Close liaison is maintained with the Colon Cancer Task Force at the Institute level.

Since the program is in its initial year, description of the background of the problem and the resources being assembled must substitute for a description of research findings.

Background: Colon cancer is basically an environmental disease, since its incidence rises dramatically in individuals who migrate from a low to a high risk area. The environmental agent has not been demonstrated to have the spreading pattern of a contagious agent. Intestinal cancer is not associated with cigarette smoking, and the one reported example of an industrial risk, namely in asbestos workers, is not yet well documented. Hence, the most likely place to search for the environmental factor is in the diet of the general population.

Adenomas of the colon show the same epidemiology as colon cancers. Adenomas are rare in Japan, but appear almost as frequently in Japanese migrants to Hawaii as in native-born Caucasian Americans. This prevalence is about 50% after the age of 40 in both groups. Hence, it is likely that the environmental factor responsible for adenoma production and for colon cancer is nearly ubiquitous in the United States. The lack of a social class gradient for large bowel cancer suggests the food item must be common rather than a luxury.

Past data from this country showed fairly large differences in bowel cancer incidence rates: North greater than South, urban greater than rural, Jewish greater than Protestant, White greater than Black, but these differences are now diminishing and in some instances (Black vs. White in the North) have vanished. Since these incidence rates reflect exposures of twenty years ago and more, it is not surprising that recent retrospective and prospective studies of fairly homogenous White populations have identified no general environmental factors associated with a high risk of large bowel cancer.

Studies of populations at different risks for large bowel cancer have shown not only great differences in diet (so far too many to point to any one item) but differences in gut physiology, flora, and fecal contents. In rodents, the pattern corresponding to the distribution of human cancers; namely, intestine, is seen only when the carcinogen is produced from a fecal precursor by action of gut flora.

Preliminary intramural work in the Epidemiologic Pathology Unit raises the possibility that such items as trace metals and vitamins could be acting as secondary influences on the incidence rate, although none is at all likely to be the primary environmental factor. In comparisons of disease rates in various countries, colon cancer also is epidemiologically associated with cancers of the breast, prostate, pancreas, ovary, and endometrium, and with coronary heart disease, diabetes, appendicitis, and varicose veins. Most of the associations are only for populations and little data exists on multiple diseases or other factors associated with relative risk differences in individuals. One exception to this is that preliminary studies of the diet histories of Japanese migrants, for the first time, have associated high relative risks with certain foods. Although this data is not yet suitable for release, it can and ought to be used in planning.

When colon cancer increased in Japanese migrants to Hawaii, colon cancer increased much more than rectal cancer. Studies here indicate that the upper half of the rectum reacted as the colon, while the incidence of cancer in the lower half of the rectum was unchanged. If this pattern can be confirmed in other populations, it would be necessary to completely alter the epidemiologic definition of the rectum.

Program Resource Description: Much of the work on the epidemiology and pathology of bowel cancer continues to be carried out in the established contracts of the Migrant Program of the Biometry Branch, Demography Area of Etiology. The studies on Norwegian migrants (Norwegian Public Health Service, NIH-NCI-E-64-499, and University of Minnesota, NIH-NCI-E-66-919) include case control interviews of bowel cancer patients. Preliminary results are becoming available and are being used in planning the Segment's work, as has unpublished data gleaned from the Norwegian Cancer Registry files by the Segment Director. The contract with Universidad de Valle, Cali, Colombia (NIH-NCI-E-66-907) has yielded data on the epidemiology of precancerous lesions of the colon as well as of colon cancer. We are asking that Dr. Correa's protocols for these studies be employed for the parallel studies in Japan, Hawaii, and New Orleans (see below). We also are planning to carry out more specific epidemiologic studies in Cali and extend the pathology studies to Medellin, Maracaibo, Caracas, Reciefe, San Jose, and Costa Rica in order to observe the changes accompanying increased affluence of groups initially at low risk of colon tumors.

The contracts with Tohoku University School of Medicine (NIH-NCI-E-64-10), California Department of Public Health (NIH-NCI-E-64-873), University of Hawaii (NIH-NCI-E-63-558) and Kuakini Hospital (NIH-NCI-E-71-2170) support the broadest mix of bowel cancer studies. Case-control diet studies are being completed. Protocol studies on the pathology of bowel cancers in Hawaii and Japan are now at the point of analysis. Studies of precursor

lesions have been set up. Arrangements are being made in Hawaii for the collection of material for our bacteriology and chemical projects from individuals with bowel tumors who will also be studied as to diet, general health, blood chemistry and bowel physiology and who come from an already intensively documented population. In Japan, the contract also supports ultrastructural studies of early bowel cancer and related lesions. The Director of the Colon Cancer Segment serves as Assistant Project Officer for all but the University of Minnesota contract, and is particularly involved in the planning and operational aspects of the bowel cancer research.

In the past year the Segment has also worked closely with staff of the Ecology Studies Section, Epidemiology Branch, Demography developing studies of bowel cancer mortality in the United States; with the End Results Section, Biometry Branch, Demography on epidemiologic pathology studies paralleling the analyses on Japanese and Hawaiian cancer data; and with the Third National Cancer Survey, Biometry Branch, Demography to develop special aspects of bowel cancer incidence data.

The Segment plans to continue to exploit these resources to the fullest and to have its own program begin where these others leave off.

The Segment operates three contracts funded by the Demography Area:

1. Louisiana State (NIH-NCI-E-71-2323). "Pathologic Study of Pre-malignant Lesions of the Colon." Data on the frequency of occurrence and location within the bowel of adenomas, hyperplasias and occult cancers is being collected on Blacks and Whites to be compared with the results reported from Colombia and forthcoming from Hawaii and Japan.

2. Virginia Polytechnic Institute and State University (NIH-NCI-E-71-2427). "Fecal Flora Studies." The most comprehensive characterization attempted to date of fecal flora (defined to species) associated with bowel tumors, high-risk populations, and specific bile excretion patterns. To the extent possible, the determinations will be carried out on material from Hawaiian Japanese but other epidemiologically defined groups may be added or substituted.

3. University of California at Los Angeles. "Fecal Flora Studies." The goals are the same as for the preceding contract but there is a different bacteriological strategy. Selective media will be used to detect organisms present in small numbers, and that may permit the isolation of "indicator organisms" characteristic of the precancerous state as well as identify members of the total population. The VPI procedure offers a quantitative and qualitative description of the total bacterial flora. The patients to be studied will be in Los Angeles. They will be identified through the California Department Health Contract program and will be given diet interviews by that group's staff.

Narratives for these contracts will be found in the Report of the Demography Area.

The following are the established Contracts of the Segment within the Carcinogenesis Area:

1. American Health Foundation (NIH-NCI-E-71-2310). "Experimental Large Bowel Carcinogenesis." This is our basic model resource. Its first task was to establish a basic model by choosing the compound, the species, and the mode of administration most suitable for a baseline. The first compound tested was 3,2'-dimethyl-4-aminobiphenyl. It was chosen on the basis of literature reports but was given up because most of the tumors appeared in the small intestine. Dimethylhydrazine now appears to be the carcinogen of choice, the rat rather than mouse or hamster the best test animal, and sub-cutaneous injection the preferred mode of administration. Skin painting and intra-rectal instillation are being tested as alternate routes. The next steps will be to establish the optimal doses for various purposes such as tests of cocarcinogenicity and to establish the system in animals with a stable, defined gut flora as well as in germ-free animals. Concomitantly, the group also is investigating the relationship of bile metabolites to bowel cancer and assisting in the planning of epidemiologic studies.

2. Cornell University Medical College (NIH-NCI-E-72-2041). "Regulatory Control of Cell Proliferation in Colonic Tissue (in Familial Polyposis)." The American Health Foundation Contract should develop, at a later stage, the capability of studying subcellular changes of carcinogenesis. However, there is an opportunity to go directly to the heart of the matter in a human system, since a single gene mutation in man produces a hereditary condition that invariably results in colon cancer: familial polyposis. In the Cornell contract we are supporting the first effort ever to determine the metabolic change in the intestinal epithelial cell that is the basis of these patients' propensity for cancer. Initial studies are focusing on enzyme systems related to nucleic acid metabolism, but other areas will be included as soon as possible.

3. Oxford University. "Determination of Disease Linkages." Most environmental carcinogens produce many effects besides cancer. Cigarettes are a good example. Hence, more and more emphasis is being placed by epidemiologists such as Denis Burkitt on associations between diseases. These are difficult to study, since hospital material is subject to well-known biases while there are geographical follow-up registries for almost no disease but cancer. One exception to this is the Oxford Record Linkage System. Arrangements have just been made to search their computer tapes for diseases related to bowel tumors, and to verify and elaborate on statistical associations that appear by record review.

The Oxford Contract is our most formal project of a general program to extract data relating to colon cancer from as many data banks as possible. For instance, we have initiated a collaborative effort with the Heart and Lung Institute to identify all bowel (and other) tumors found in the Framingham, Massachusetts study population, and to characterize the individuals developing such tumors. This supplements a similar collaboration in Hawaii between the Heart and Cancer programs and, hopefully, will be extended to other groups of people being intensively studied by NIH.

Importance of the Segment Program: Large bowel cancer is, except for skin cancer, the most common cancer afflicting the U.S. population. Only lung cancer causes more cancer deaths. The NCI migrant program has helped establish that bowel cancer is caused by an environmental factor and promises to be the first epidemiological investigation to throw suspicion upon particular diet items as causal factors. Moreover, the migrant cohorts are optimal for testing hypotheses advanced by others. The Segment hopes to mobilize the resources and diverse scientific talents necessary to follow up the findings of the migrant epidemiologic studies and to provide related base-line information.

Future Plans: In addition to the programs included in the six contracts, it is hoped to include the following:

1. Develop a program at Fort Detrick to study bacteriologic metabolism of materials suspected of being precarcinogens in the human system, particularly in mixed cultures.

2. Test bile metabolites and other unevaluated suspect items for carcinogenicity in general and colon carcinogenicity in particular.

3. Organize biologic and epidemiologic studies in appropriate non-migrant populations to confirm (or disprove) associations found in migrants and to test other relevant hypotheses.

4. Modestly extend the studies of the associations between bowel cancer and other diseases or other special patient characteristics.

CONTRACT NARRATIVES

AMERICAN HEALTH FOUNDATION (NIH-NCI-E-71-2310)

Title: Experimental Large Bowel Carcinogenesis

Contractor's Project Director: Dr. Ernest Wynder

Project Officer (NCI): Dr. John Berg

Objectives: To establish suitable animals models of colon carcinogenesis; to study colon carcinogenesis in bacteriologically defined and controlled systems; to determine the activity of dietary factors as modifiers of colon carcinogenesis; to determine the carcinogenicity of selected bile metabolites; and to carry out parallel studies in animal and man relating to the role of diet in general and bile and tryptophane metabolites in particular in colon carcinogenesis.

Major Findings: Work began before this unsolicited proposal was first funded. As a result, it has already been determined that the original compound chosen for the model system (3,2'-dimethyl-4-aminobiphenyl) is not appropriate (too many small intestinal cancers). No effects of diet were noted. Dimethylhydrazine has proved much more satisfactory. Similarly, tests in three species have shown that rats are the best

for our purposes. (Hamsters on DMBA unexpectedly developed bladder tumors. Since there are major advantages in direct contact carcinogenesis, intrarectal application of several agents is being studied. To date only N-methyl-N -nitroso-N-nitrosoquanidine has produced turmors. In the initial studies of fecal chemistry, it has been found that Chinese-Americans have a quite different profile of sterols and bile acids than either Japanese-Americans or Caucasian-Americans.

Significance to Biomedical Research and the Program of the Institute: While the proposal was unsolicited and represents Dr. Wynder's considered program to link animal experimentation and the study of human cancer causation, the project also serves as the Segment's central resource for non-bacteriological laboratory investigation. Of particular importance are the skills of the supporting staff (Reddy and So) and the overall flexibility, so badly needed at this stage of the Segment's program.

Proposed Course: (1) To determine an optimal regime of DMH carcinogenesis for rapid results and sensitivity to modifying factors; (2) to standardize the methodology for intrarectal carcinogenesis; (3) to study these systems in germfree and defined flora rats; (4) to continue studying fecal chemistry in populations of epidemiologic interest with regard to colon cancer, to relate the results to food intake and to translate findings back to animal model work; (5) to test the carcinogenicity of bile metabolites in the mouse skin induction promotion system.

Date Contract Initiated: June 30, 1971

Current Annual Level: $350,000

CORNELL UNIVERSITY MEDICAL COLLEGE (NIH-NCI-E-72-2041)

Title: Regulatory Control of Cell Proliferation in Colonic Tissue (in Familial Polyposis)

Contractor's Project Director: Dr. Martin Lipkin

Project Officer (NCI): Dr. John Weisburger

Objectives: To determine the enzymatic and/or kinetic changes that characterize the earliest stages of the pathological bowel mucosa of familial polyposis and so to determine the basic molecular lesion initiating carcinogenesis in this condition inherited through a dominant gene.

Major Findings: None yet available because of the short duration of the contract. Investigations began first on enzyme inactivation since these analyses were being done on non-polyposes. Studies of the enzymes involved in nucleic acid formation and of cell repair mechanisms are being established.

Significance to Biomedical Research and the Program of the Institute: Studies have established that an environmental or cultural change in Hawaii must be responsible for the increased colon cancer found in Japanese Migrants to the United states compared to Japanese in Japan. The major effort relating to colon cancer in the Etiology Area is the attempt to discover what this factor is. One major handicap is the long period (perhaps 20 years) between exposure and development of cancer. The period can be shortened and the number of index cases increased by using adenomas rather than carcinomas to mark index cases but even so the time gap is substantial and the number of cases still small.

There is every reason to believe that the adenoma-cancer transition is the same in multiple polyposis as it is in colon cancer generally. It seems quite possible that the earlier stages of tumorigenesis might also be the same. If so, the polyposis syndrome, caused by a single gene mutation would be a most productive place to look for the earliest possible marks of carcinogenesis. Also, by identifying the underlying molecular alteration, we could be a great deal closer to determining what type of environmental factor we are looking for. It is submitted that genetic defects of this type have been the most fruitful source of our understanding of the molecular pathology underlying human disease and that our study of colon cancer should take advantage of the existence of this appropriate "experiment in nature."

Proposed Course: As metabolic peculiarities of the colonic epithelium of polyposis patients are identified, explanations will be sought further back in the metabolic chains until the initial defect is identified.

Date Contract Initiated: October 15, 1971

Current Annual Level: $115,400

SUMMARY REPORT

INFORMATION AND RESOURCES SEGMENT

Fiscal Year 1972

The Information and Resources Segment has two major areas of responsibility within the Carcinogenesis contract program. The first of these requires it to provide support to other segments by assuming responsibility for the direction and management of contracts which are essential for the continued functioning of that segment's activities but for which no particular expertise is available within that segment's working group. An example of such an activity lies in the responsibility by this Segment for the contract under which particulate materials are prepared for use as a resource in contracts conducted within the Lung Cancer Segment. Such support is essential since the sorts of knowledge and skills required to provide continuing review and direction to the overall Lung Cancer Program are quite different from those which are needed to direct and manage a program in the area of fine particle technology. It is in just such areas that the chemical expertise available in the I&RS working group can best support the overall program.

The second function of the Segment lies in the anticipation, planning, and provision for services which meet the resource needs of the total Carcinogenesis Program as distinct from the needs of specific segments. Such activities are exemplified by the production of the journal, Carcinogenesis Abstracts, and its distribution to that part of the scientific community having an interest in the problems with which we are concerned. In general the types of needed resources appear to have fallen naturally into three categories, the first of these being animal resources, the second chemical resources, and the third information.

The Information and Resources Segment has been established during this fiscal year. Drs. J. A. Cooper and S. Siegel served as Segment Director and Manager, respectively. A working group has been established composed of NCI personnel having expertise in specific areas of concern. This staff group includes Drs. D.A. Kaufman, N.P. Page, and Mr. R. Newell in the area of animal resources, Drs. J. A. Cooper, L. Keefer, and E. K. Weisburger in the area of chemical resources, Dr. S. Siegel and Mr. T. Kuch to provide expertise in the area of information sciences. The Segment Advisory Group which includes six members of the scientific community external to NCI has been established. Since even such an expanded working group will contain a very limited number of chemists or information scientists, it will continue to be necessary for us to draw on other members of the scientific community on an ad hoc basis to assist in the evaluation of individual proposals, in the conduct of site visits and in the long range planning of program resource needs. Such a program of involvement of scientists from outside the Carcinogenesis Area is now under way and, in fact, in this fiscal year sixteen persons have been utilized on such an ad hoc basis, twelve of them being from outside of the NCI.

It is projected that in this fiscal year the Segment's activities will

include some twenty-two contracts and a total budget of approximately two million dollars. About half of these funds will be expended in the area of information services, the greater part of the remainder will be devoted to chemistry resources with a relatively small level of activity in the animal resources area.

CONTRACT NARRATIVES

ASH STEVENS (NIH-NCI-E-66-929)

Title: Synthesis of Purine and Pyrimidine Nucleotides

Contractor's Project Director: Dr. Calvin Stevens

Project Officer (NCI): Dr. Michael Sporn

Objectives: The general aim of this contract is to design and make available a new type of pharmacological agent which will be useful in investigating the manner in which chemical and viral carcinogens affect the metabolism of newly synthesized and messenger RNA in the cell nucleus, as well as a variety of other problems in chemical and viral carcinogenesis. The specific aim of the contract is to synthesize an organic chemical which will irreversibly inactivate the nuclear enzyme which destroys newly synthesized and messenger RNA in the nucleus itself; the chemical agent must be selective and biologically active. An additional aim of this contract has been added since its inception: during the site visit of January 13, 1971, it was strongly suggested by Professor Gerald Mueller, McArdle Laboratory for Cancer Research, University of Wisconsin, that compounds which have been synthesized under this contract be assayed for possible inhibition of the RNA-dependent-DNA-polymerase (reverse Transcriptase) of onogenic viruses and leukemic cells. This aspect of the project has been implemented during the past year and is now yielding important results.

Major Findings: Several new agents have been synthesized and tested during the past year and have been found to be potent inhibitors of both nuclear exoribonuclease and reverse transcriptase. The reverse transcriptase assays have been performed by Dr. Anthony Schrecker and Dr. Robert Gallo, NCI. The most active agents have been thymidine-3',5'-di-m-bromoacetamidophenylphosphate (TDMB) and 2'-O-methyl uridine-3',5'di' m-bromacetamidophenylphosphate (Um DMB), both of which give significant inhibition of exoribonuclease at 10^{-5} Molar concentration, and of reverse transcriptase at 10^{-6} Molar concentration. The synthesis of Um DMB represents a first in organic synthesis, namely the first use of a 2'-O-methyl nucleoside in the synthesis of an active-site-directed enzyme inhibitor.

Significance to Biomedical Research and the Program of the Institute: Within the past few years, there has accumulated an important body of data which show that in many different vertebrate cell types, most of the RNA that is made in the cell nucleus is destroyed in the nucleus itself very shortly after synthesis. The figures that have been published indicate

that as much as 90% of the newly synthesized RNA is destroyed in the nucleus itself, and that its half-life may be only a few minutes.

The entire field is stll virtually untouched with respect to the problem of carcinogenesis. It is well established that chemical carcinogens have immediate and profound effects on the metabolism of RNA in the cell nucleus. The development of a selective drug probe, which would enable the investigator to stop the destruction of messenger RNA in the cell nucleus (without interferring with its synthesis), would open up a wide variety of experimental work in the field of chemical or viral carcinogenesis.

Moreover, the synthesis and testing of potential inhibitors of reverse transcriptase, a field in which this contract has already yielded successful results, is an area of high priority and relevance in other aspects of the NCI total program.

Proposed Course: To continue to develop new synthetic nucleotide analogs that will have greater biological potency and selectivity. To continue to test these nucleotide analogs in carcinogenesis test systems, such as their effects on malignant transformation of cells by Rous sarcoma virus, as well as for their effects on the RNA-dependent-DNA polymerase (reverse transcriptase) of oncogenic viruses and transformed cells.

Date Contract Initiated: June 21, 1966

Current Annual Level: $137,280

FRANKLIN RESEARCH INSTITUTE, LABS. (NIH-NCI-E-71-2073)

Title: Carcinogenesis Abstracts, Vol. IX

Contractor's Project Director: Mr. Alec Peters

Project Officer (NCI): Dr. Sidney Siegel

Objectives: The publication of this abstract journal in the field of carcinogenesis provides an up-to-date abstract service for carcinogenesis abstract research through out the world in a format designed to provide under one cover scientific work in the various disciplines which relate to cancer etiology.

Major findings: This contractor has performed well and during the period has developed the required product on schedule. The contractor has noted an increase in information which can be considered relevant for this publication.

Significance to Biomedical Research and the Program of the Institute: The multidiscipline approach used in the study of carcinogenesis requires information source which pulls together relevant articles under one

publication cover. This allows exchange of information and ideas among the disciplines which comprise carcinogenesis research.

Proposed Course: (1) To continue the publication for an indefinite period of time. (2) To make a determination at the completion of Volume 10 as to whether or not this publication should be completed commerically. (3) To determine with the contractor the accuracy of the apparent increase of relevant information.

Date Contract Initiated: January 7, 1971

Current Annual Level: $44,200

FRANKLIN RESEARCH INSTITUTE, LABS. (NIH-NCI-E-72-2040)

Title: Preparation of Carcinogenesis Abstracts Vol. X

Contractor's Project Director: Mr. Alec Peters

Project Officer (NCI): Dr. Sidney Siegel

Objectives: The publication of this abstract journal in the field of carcinogenesis provides an up-to-date abstract service for carcinogenesis researchers throughout the world in a format designed to provide under one cover scientific work in the various disciplines which relate to cancer etiology.

Major Findings: This contractor has performed well and delivered the required product on time. A careful monitoring of the literature by the scanning and consulting staff of the contractor has demonstrated a 20% increase in relevant information since the contract was let.

Significance to Biomedical Research and the Program of the Institute: The multidiscipline approach used in the study of carcinogenesis needs an information exchange medium which gathers the relevant articles under one cover and once done allows easy intellectual cross fertilization among the disciplines to produce a more rapid evaluation of information in the study of carcinogenesis.

Proposed Course: (1) To continue the publication for an indefinite period of time. At the end of Volume 10 a decision will be reached as to whether or not the abstract journal should be commercially produced. (2) To work out with the National Cancer Institute a mechanism by which the information processor can catch up with the increasing amounts of relevant information.

Date Contract Initiated: November 1, 1971

Current Annual Level: $54,000

IIT RESEARCH INSTITUTE (NIH-NCI-E-70-2245)

Title: Production and Characterization of Particulate Materials for Studies in Respiratory Carcinogenesis

Contractor's Project Director(s): Mr. Reg Davies

Project Officer(s) (NCI): Dr. John Cooper

Objectives: As required by the National Cancer Institute in its Respiratory Carcinogenesis Program to do research into both the methodology and efficiency of coating particles with selected polynuclear hydrocarbons and then storing them in a manner to prevent deterioration in quality; specifically to prepare and characterize the following materials: (1) powder fractions, differing in size, composition and surface area; (2) powder fractions coated with selected polynuclear hydrocarbon (3) dispersions of coated particles in selected vehicles; and (4) stable emulsions of selected polynuclear hydrocarbon carcinogens in selected vehicles.

Characterization of the samples includes the following determinations: (a) purity of the sample as determined by emission spectroscopy; (b) the actual particle size distribution of the sample; (c) information on the conformation of particles in the sample as determined using the scanning electron microscope; (d) a surface area measurement performed on the sample by inert gas absorption.

Major Findings: (1) Particle Preparation - Gram quantities of carbon, ferric oxide, alumina, nickel oxide, and cobalt oxide have been prepared in four size ranges with 90% of the particles falling within the specified limits of 0.5-1.0 microns, 2-5 microns, 5-10 microns and 15-30 microns. The cascade sedimentation method was used. Fractions of these materials are in use in respiratory studies.

(2) Particle Coating - Particles of carbon, hematite and alumina have been coated with benzo(a)pyrene (BP) and have been supplied to the National Cancer Institute, Massachusetts Institute of Technology, Ohio State University, and IIT Research Institute's Life Sciences division. Over 60 coating preparations have been made to date, and on each sample photomicrograph characterization for free BP and the degree of agglomeration has been made.

(3) Dispersion of Particles in Liquids - Due to the sedimentation instability of BP-coated particles in gelatin-saline vehicles during transport, all coated particles have been supplied as dry powders. In all cases, samples of control dust have been submitted at the same time.

(4) Preparation of Emulsions - In some programs, BP was administered as a ball-milled preparation (emulsion) in the presence of gelatin and saline. Various BP loadings were prepared as requested, approximately 40 preparations have been made. All of these preparations have been characterized with respect to the BP content and particle size distribution.

The period of time the coated particle has remained usable has increased as a result of a study involving storage temperature. BP needle formation has been greatly reduced with the storage environment maintained at-70°C.

Significance to Biomedical Research and the Program of the Institute: This effort is intended to produce the particulates required for the execution of other contracts in the Carcinogenesis Program. In addition, this effort will provide investigative and production support for such contracts, in the areas of fine particle technology and stable emulsion preparation, characterization, storage and thus can supply a closely controlled species of both coated and uncoated particles.

Proposed Course: It is intended that this contract be continued as a production and service resource for related contract activities in this area. It is anticipated that the number of preparations made for the various respiratory carcinogenesis programs will increase from 1,000 to 1,500 in Fiscal Year 1973.

The workscope for the coming support period will include: (1) The preparation and characterization of the 20 powder fractions will continue. (2) The preparation of coated fractions of particles will continue. Here, it is expected that other carcinogens will be introduced. (3) The preparation of suspensions of particles in various vehicles will proceed and will increase. In particular, long-term studies using cobalt and nickel oxides are planned. (4) Research studies into better mechanisms of powder coating will be initiated, as this problem has been acute during recent studies. And (5) A study into the degradation rate of carcinogen in all preparations is contemplated. It is essential that the extent of degradation and actual concentration of BP be measured after preparation and normal storage.

Date Contract Initiated: June 10, 1971

Current Annual Level: $98,565

MASSACHUSETTS GENERAL HOSPITAL (NIH-NCI-E-71-2128)

Title: Atlas on Comparative Morphology and Classification of Spontaneous Neoplasms in Dog, Cat and Man

Contractor's Project Director(s): Dr. Alan Schiller

Project Officer(s) (NCI): Dr. Norbert Page

Objectives: To create a reference atlas on spontaneous and induced tumors encountered in the dog and cat, together with information on their histopathology, epidemiology, and etiology, with emphasis on comparative aspects of similar neoplasms of man.

Major Findings: During the first year of this contract there were two major achievements: (1) The autopsy and hospital populations have been obtained and tabulated to allow for correlations of frequency, distribution and occurrence of specific neoplasms; (2) Chapters on respiratory tract and endocrine neoplasms are nearing completion while the study of tumors of the central nervous system has been initiated.

Significance to Biomedical Research and the Program of the Institute: The projected use of this book would be to provide (1) a consolidated morphological and biological description and illustration of spontaneous neoplasms in the dog and cat with an accompanying bibliography and standardized nomenclature, (2) a description of new spontaneous and experimentally produced canine and feline tumors heretofore unreported, and (3) comparative study with human neoplasms for use in selection of animal models for research.

Proposed Course: This is a cooperative project between human pathologists at the Massachusetts General Hospital and veterinary pathologists at the Angell Memorial Animal Hospital. It is expected that this project will be completed in June 1974 (3 years). During the first two years, the major emphasis will be on performing the histological examinations and obtaining data from case histories, etc. The final year will be devoted to the collation of these data and making the comparisons of animal and human tumors.

Date Contract Initiated: May 1, 1971

Current Annual Level: $55,000

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NIH-NCI-E-70-2180)

Title: Environmental Occurrence of N-Nitroso Compounds

Contractor's Project Director(s): Dr. Steven Tannenbaum
Dr. Gerald Wogan

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: The interaction of nitrite with substituted nitrogen compounds can lead to a series of compounds containing the N-nitroso residue. Reports of the presence of nitrosamines in several foods and beverages raises the question of their significance in the etiology of human cancer. The contractor is therefore developing analytical procedures for nitrosamines in foods and beverages. Since nitrite has been shown to be fairly ubiquitous in the environment, it is important to know which conditions favor its reaction with a specific nitrogen compound. Therefore, studies are also being conducted to define the reaction kinetics in foods and model systems and identify reaction promoters and retardants.

Major Findings: Methodology has been streamlined for the isolation and analysis of volatile nitrosamines utilizing the Coulson Electrolytic Conductivity Detector as a specific N-detector for gas chromatography. Clean-up procedures have been refined to enable analyses of a variety of types of cured meats. Procedures have also been developed to analyze alcoholic beverages using a Freon extraction system. The method for automatic analysis of nitrites and nitrosamines has been further refined and has been applied to alcoholic beverages and saliva. The finding of high, constant concentrations of nitrite in human saliva would appear to be of major significance. Studies have also been made on the formation and decomposition of certain nitrosamines. The extraordinary accelerating effect of thiocyanate on amine nitrosation has been quantitatively described.

Significance to Biomedical Research and the Program of the Institute: Since N-nitrosamines have proved to be multipotential carcinogens in all animal species tested, they may represent significant hazards to man as environmental contaminants contributing to the "spontaneous" cancer incidence. This project will aid in determining the total extent to which nitrosamines may be present in the environment and the conditions favoring their formation during processing and storage. Thus measures to prevent this formation could be instituted.

Proposed Course: The project will continue along the same line, attempting to refine analytical techniques for nitrosamines and determining conditions favoring their formation. Additional emphasis will be placed on analysis of foods and beverages from geographical regions having a high incidence of cancer.

Date Contract Initiated: June 11, 1970

Current Annual Level: $75,000

MEDIZINISCHE HOCHSCHULE--HANNOVER (NIH-NCI-E-71-2184)

Title: Development of a Lung Tumor Model with Large Wild Hamsters (Cricetus cricetus)

Contractor's Project Director: Professor Ulrich Mohr

Project Officer(s) (NCI): Dr. Norbert Page

Objectives: This contract was initiated to explore the possible usefulness of the common wild European hamster (Cricetus cricetus) as an animal model for bronchogenic keratinizing squamous cell carcinoma of the lung similar to human lung cancer. The specific aspects are to (1) capture the wild hamsters and establish a breeding colony, (2) determine the incidence of spontaneous tumors especially brochogenic carcinoma, and (3) test for sensitivity to induction of lung cancer by known carcinogens.

Major Findings: Preliminary findings indicated that this species of hamster had a high incidence of keratinizing bronchogenic squamous cell carcinoma if they were obtained from an industralized area of West Germany. It was not clear whether this species was highly sensitive to cancer of the lung induced by environmental pollutants or had a high natural spontaneous incidence.

Hamsters were easily obtained from a rural area, have been adapted to the laboratory and have been bred successfully. An F-generation is now being raised for further development of a breeding colony.

Eight known carcinogens are being tested in the wild-caught animals. LD_{50}'s and tolerated doses have been determined and many of the chemicals are now being administered in chronic studies. Chronic treatment with N-diethyl-nitrosamine in a small study resulted in all animals (10) dying with keratinizing squamous papillomas of the nasal and paranasal cavities. Two had bronchogenic squamous cell carcinoma. Tumors were also found in several other areas.

Signigicance to Biomedical Research and the Program of the Institute: Regardless of the causation of the squamous cell carcinoma, having available laboratory animals with this characteristic would be invaluable to cancer research. Other characteristics which would make this animal superior to the Syrian golden hamster, are its larger size and its longer lifespan. At present there is no satisfactory animal model for study of the natural pathogenesis of squamous cell carcinoma.

While this type of tumor can be induced in the Syrian golden hamster via intratracheal instillation of chemical carcinogens, they do not spontaneously develop these tumors. Consequently, it is necessary to have reservations in extrapolating data obtained with the Syrian hamster to man. In addition, the use of an animal that is subject to the spontaneous occurrence of squamous cell carcinoma, might be more suitable to studies of lung cancer using a natural exposure method, i.e., inhalation of aerosols.

The program is of direct and intimate relevance to the lung cancer program. If this species can be bred and maintained as a laboratory animal, it would be a valuable animal for the bioassay programs as well as to study mechanisms and pathogenesis of spontaneous lung cancer.

Proposed Course: It is expected that this project will be completed in June, 1974 (three years). Initially it was necessary to capture and adapt this wild species to laboratory conditions and develop a breeding program. The natural occurrence and incidence of diseases, expecially lung tumors, has to be determined.

The effort to obtain hamsters from an industrialized area has not been successful but will be continued during the next year. The chronic testing of known carcinogens by the intratracheal instillation method will be continued. As the project progresses, studies on inhalation of carcinogens will be conducted as determined by NCI program needs.

Date Contract Initiated: June 22, 1971

Current Annual Level: $34,150

MIAMI, UNIVERSITY OF, (NIH-NCI-E-71-2274)

Title: Search for Possible Plant Causes of Esophageal Cancer

Contractor's Project Director: Mrs. Julia Morton

Project Officer (NCI): Dr. John Cooper

Objectives: This project is designed to identify potential carcinogens which may be responsible for the high incidence of esophageal carcinoma in humans in many parts of the world, and thus to help explain why the distribution of esophageal cancer is unexplainably irregular and is often high in one region or portion of a country while adjacent regions are low.

Major Findings: On Curacao in the Dutch West Indies there is an incidence of esophageal cancer of 20-23 new cases annually in a population of 140,000. Previous investigations by this contractor have revealed widespread use of bush teas by the general population including those developing esophageal cancer. Several of these teas have been shown (by members of the National Cancer Institute Staff) to induce sarcomas in rats, especially Krameria ixina which is known to be rich in catechin tannin. An effort was made to discover a factor common to Curacao and to other portions of the world where esophageal cancer is high. A search of the Morton Collectanea revealed the use of tannin-containing food and beverages in many regions where the incidence of esophageal cancer was high. Epidemiological data on the esophageal-cancer-prone population of the South Carolina coastal region have been gathered. While the incidence of esophageal cancer is high in thelow areas, further investigation is needed to determine possible etiological associations.

Significance to Biomedical Research and the Program of the Institute: The aims of this project are in direct line with the basic objectives of the carcinogenesis program, i.e., to identify potential carcinogens in man's environment and to test these elements experimentally in the laboratory. Once a factor has been confirmed as hazardous to man, ways can be found to eliminate it or to minimize its influence.

Proposed Course: The studies on Curacao and South Carolina will be continued and expanded by investigation of the occupational histories of esophageal cancer patients (especially regarding leather and wood-working), by inquiries into the consumption of tea and high tannin Portuguese red wines, and by further collection of suspected plant materials for bioassay and tannin analysis.

Date Contract Initiated: June 30, 1971

Current Annual Level: $53,384

SOUTHWEST RESEARCH INSTITUTE (NIH-NCI-E-72-2065)

Title: Development and Application of Analytical Methods of Volatile Nitrosamines in Complex Mixtures

Contractor's Project Director(s): Dr. Don Johnson

Project Officer(s) (NCI): Dr. John Cooper

Objectives: The objectives of this contract are to develop methods for the analysis of smoke condensate to apply these methods to determine potentially harmful chemicals and more specifically the development and application of methodology for the analysis of N-nitrosamines. Emphasis has been placed on tobacco smoke as a potential source of N-nitrosamines. The overall objective of the contract is to identify and measure those compounds which contribute to the total carcinogenic activity of tobacco smoke. The method should also be useful for application to other samples such as food products.

Major Findings: During the preceding contract year, N-dimethylnitrosamine was identified in cigarette smoke condensate. Methylethylnitrosamine was tentatively identified. It was shown that the growing conditions of the tobacco, especially soil nitrogen, significantly affected the amounts of these two nitrosamines produced in the burning of the tobacco in cigarettes.

Significance to Biomedical Research and the Program of the Institute: In order to reduce the harmful aspects of smoking, it is essential to identify and measure each of the toxic substances. This program in identifying in cigarette smoke condensate a class of chemical carcinogens has provided a method suitable for quantifying their concentrations and thus become able to monitor attempts to remove them from the condensate.

Proposed Course: The program will continue to define those parameters of tobacco usage which alter the production of nitrosamines and establish where in the series of events from combustion through collection of the products of combustions are the nitrosamines formed.

Date Contract Initiated: December 23, 1971

Current Annual Level: $89,347

STANFORD RESEARCH INSTITUTE (NIH-NCI-E-71-2045)

Title: Retrieval and Ranking of Potential Carcinogenic Hazards

Contractor's Project Director: Arthur A. McGee

Project Officer (NCI): Dr. Gio Gori

Objectives: Define and demonstrate the feasibility of a systematic methodology for information retrieval and ranking of estimated risk for potential chemical carcinogenic hazards to man.

Major Findings: In November 1970, the National Cancer Institute commissioned Stanford Research Institute to exert its best efforts to define and demonstrate the feasibility of a systematic methodology for information retrieval and ranking of estimated risk for potential chemical carcinogenic hazards in man (NCI Contract NIH-71-2045). The basic approach used by the research team has been to:

1. Develop a general methodology for arriving at a hazard ranking for chemical compounds found in the environment.

2. Demonstrate the methodology by applying it to representative categories of human exposure and groupings of chemicals.

3. Extrapolate the results of the demonstration over the universe of consideration to assess feasibility.

The parameter used in the ranking methodology is the estimated total hazard presented by a chemical. Hazard is taken to be a function of both man's exposure to the chemical and the degree of suspicion of carcinogenic activity and potency that can be associated with it.

The objective of the quantification of human exposure is to arrive at an estimate of the intake of all chemicals for each significant segment of the population. The basic approach is to conduct two interactive analyses tracing chemicals forward from their point of origin to their ultimate disposition and also tracing them backward through product types that men are exposed to from the standpoint of use. This two fold approach ensures that all quantities produced are accounted for, that impurities and contaminents are not overlooked, and that detail is added only when it is actually needed.

Exposure modes are classified so as to take advantage of available statistics on production, distribution, and human intake. The top level categories are: Household, environmental, occupational, and avocational. These exposure categories are further subdivided by major and minor use classes and then by product type. Each product type is characterized by functional classes of compounds and within each class by specific chemicals. Next a composite formulation is determined reflecting the quantities of each chemical by functional class. Intake estimates by route (i.e. oral, inhalation, cutaneous) are arrived at by applying attenuation factors reflecting the actual conditions of use, length of exposure, concentrations, and similar data.

To minimize the cost of obtaining exposure estimates, a first estimate is made based upon an upper limit of intake. If the compound ranks high on the hazard list the estimate is subsequently refines based upon the expected value of intake rather than the upper limit. It is anticipated that perhaps three levels of selective refinement may be necessary before a stabilized hazards listing of chemicals is produced.

In arriving at exposure estimates, the importance of identifying small groups of highly exposed people has been made by the NCI staff and others who have acted as consultants to the project. The importance of these groups lies in their visibility; the opportunity for prospective or retrospective epidemiological surveys to confirm the hazard ranking eventually to be assigned by the system; and the great potential for prevention of cancer that control measures have in these subpopulations

The need for adequate documentation of the rationale and data sources used to arrive at exposure estimates has been stressed. This documentation will reassure the users of the system that there is a valid basis for the estimates. It will also establish the assumptions under which the estimates were made so that as new knowledge is gained, revised estimates can be inserted into the system based upon a revised set of assumptions.

The methodology just described has been applied to six functional groups of products within the four modes of exposure to demonstrate feasibility. These investigations have given us good insight into ways of obtaining exposure estimates efficiently and provided a vehicle for extrapolating the results over all exposure modes in our feasibility assessment.

The quantification of suspicion of carcinogenic activity makes use of all available biological information to provide a hazard ranking. In general, two types of information have been used: (1) generalities based on chemical structural similarities to previously tested chemical carcinogens, and (2) specific results from biological observations (other than long term chromic testing in animals) which may be useful in determining potential carcinogenicity.

A methodology has been developed to assign the probability of a substance being a carcinogen on the basis of its chemical structure. As part of this estimation process, the relative carcinogenic potency of substances is also assessed.

This methodology takes the form of estimating a probability for a general group of compounds which are structurally similar, based on those members of the group that have been tested. Examples of such groups are nickel salts, dialkyl-substituted aromatic amines, Y-lactones, and p-substituted azo dyes.

Essential to obtaining these estimates is the judgmentally weighted use of all available information. We have, therefore, called upon the knowledge and judgment of experts who are prominent in the field of chemical

carcinogenesis to assist us in providing these estimates. These experts have also been invaluable in pointing out improvements in the general technique and clarifying definitions of the concepts used in the estimation process.

With the large number of compounds that must be considered in the ranking system, computerized methods must be employed to aid in processing data. Emphasis has therefore been placed on developing and exploring the utility of existing computerized methods for handling chemical structure and substructure information. The configuration of a data base to support the requirements of an operational system was also explored. The activity tree in a preliminary form has been programmed so that we have been able to test it against populations of chemicals that are in machine-readable form. The two populations of compounds that will be analyzed in relation to the activity tree as part of this year's work are:

(1) Compounds that are produced in commercial quantities greater than 1,000 lb. or $1,000 per year--approximately 10,000 compounds, as listed in SRI's Directory of Chemical Producers.

(2) Chemical substances of interest to the National Library of Medicine and the Food and Drug Administration as shown in the Chemical Abstract Service Common Data Base. It includes drugs, veterinary medicines, dyes, cosmetics, farm chemicals, and detergents.

For each of these populations an analysis will be made to determine the number of compounds falling into each branch of the activity tree (i.e., the number for which suspicion estimates can be made) and the number for which it is not possible to make an activity estimate based upon existing experimental evidence).

As part of our first year's work, we have devoted some time to a critical review of possible criteria for suspicion other than animal test results. The possible uses of such phenomena would be to help in the assignment of probability of suspicion estimates. Biological observations that have been considered as possible predictors of carcinogenic potential are mutagenicity, immunosuppression activity, and cytotoxicity. The work this year has taken the form of attempting to determine if statistical correlation exists and to what degree.

Based upon the above investigations, the feasibility of the system has been assessed. The criteria used for this assessment have been: the logical completeness of the methodology; the practicability of the methodology for obtaining and processing system inputs; the availability of data to support the methodology; the utility of by-products of the system; and the cost of developing and operating the system. Based upon these criteria, the project team believes that the development and operation of the Cancer Hazard Ranking and Information System is feasible.

Significance to Biomedical Research and the Program of the National Cancer Institute: The system developed through this study will be useable in identifying and ranking those chemical compounds that pose the greatest suspected hazard to humans for use in guiding NCI's carcinogenic bioassay program. The methodology will represent the best knowledge available within the scientific community. Possible byproducts of the study will be to identify areas where additional information regarding chemical activity of human exposure will enhance the effectiveness of the bioassay testing program.

Proposed Course: To continue the project as outlined above or as recommended by the project officer.

Date Contract Initiated: October 30, 1970

Current Contract Level: $400,000

THOMPSON, JOHN I. (COMPANY) (NIH-NCI-E-69-2086)

Title: Literature Search and Retrieval of Data Relating to Chronic Tests of Chemicals in Animals

Contractor's Project Director(s): Mr. John King

Project Officer (NCI): Dr. Sidney Siegel

Objectives: Search the 1961-1969 literature and select articles containing data on the testing of chemicals in animals for carcinogenicity; extract the data; quality control and editing of the data extracts; arrangements of the data within 12 chemical groups in alphabetical order by CAS name; preparation of a cumulative chemical name index including all compounds in previous volumes of PHS Publication No. 149, generation of five biomedical indexes, and repro copy production for a 1968-1969 and a 1961-1967 volume of PHS Publication No. 149.

Major Findings: The 1968-1969 volume is complete. It has been printed and is in the process of being distributed. Work is proceeding essentially on schedule for the production of the 1961-1967 volume and will be available for distribution in November, 1972.

Significance to Biomedical Research and the Program of the Institute: PHS Publication No. 149 is a series of books which afford direct access to the carcinogenicity data content of the world biomedical literature. The 1961-1967 and 1968-1969 volumes fill the requirements for surveys of the literature published during these years.

This effort is consistent with the structure and goal orientation of research supported by the Carcinogenesis Program; there is constant need for documentation of work in the field. PHS Publication No. 149 is a valuable reference that serves in not only the dissemination of

scientific information but also the avoidance of research duplication. Response to initial issues of the series and more importantly to the absence of supplements covering 1961-1969 has underlined the acute need for this tool.

Proposed Course: This contract effort will end when the 1961-1967 volume is presented to the National Cancer Institute.

Date Contract Initiated: June 9, 1969

Current Annual Level: $71,554 for a six month period (October 8, 1971 through April 8, 1972

THOMPSON, JOHN I. (COMPANY) (NIH-NCI-E-71-2266)

Title: Literature Search and Retrieval and Compilation of Data Relating to Chronic Tests in Experimental Animals

Contractor's Project Director: Mr. John King

Project Officer (NCI): Dr. Sidney Siegel

Objectives: To compile and publish data from the 1970-1971 scientific literature on the carcinogenic activity of chemicals in experimental animals. Data will be arranged in 12 chemical groups. The resultant publication will be the 1970-1971 Supplement of PHS Publication No. 149, formerly entitled Compounds Which Have Been Tested for Carcinogenic Activity.

The Contractor will search the scientific literature, select appropriate documents, extract specific data from these documents, and index the documents with respect to selected items for the extracted data. Indexes will be based on the following (1) literature reference, (2) route of treatment administration, (3) site of treatment administration, (4) animal species/strain, (5) tumor site, (6) vehicle in treatment, (7) chemical names of compound administered, (8) CAS registry number of compound administered, (9) molecular formula of compound administered, and (10) Wiswesser Line Notation of the compound administered.

The chemical names, CAS registry number, molecular formula, and Wiswesser Line Notation indexes will be cumulative with respect to the entire PHS Publication No. 149 Series.

Major Findings: This contract represents a continuation of the work being carried out under NIH-69-2086, in which a 1961-1967 Supplement and a 1968-1969 Supplement to PHS Publication No. 149 are being prepared.

Literature screening and article selection are complete, data extraction, data extract editing and preparation of the cumulative indexes are in

preparation and all tasks are on schedule.

Significance to Biomedical Research and the Program of the Institute: The 1970-1971 Volume of PHS Publication No. 149, which will be produced under this contract, will bring the series of volumes up-to-date and will provide a valuable tool to be used in searching the chemical carcinogenesis literature. The cumulative chemical name, Wiswesser Line Notation, CAS Registry Number, and molecular formula indexes will enhance the usefullness of the entire series.

Proposed Course: Delivery of copy ready for publication will cause this kind of literature-gathering to be temporarily halted.

Date Contract Initiated: June 9, 1971

Current Annual Level: $79,230

SUMMARY REPORT

LUNG CANCER SEGMENT

Fiscal Year 1972

The Lung Cancer Segment, established in this fiscal year, is devoted to the study of the pathogenesis of lung cancer and its inhibition. A spectrum of experimental techniques is used in many investigations involving long-term animal studies, as well as short-term morphological and biochemical studies coordinated to establish a correlation between animal models and human lung cancer and to develop a targeted approach to the inhibition or prevention of this most prevalent form of cancer in man. The major accomplishments of this program are described below.

In the Oak Ridge "Co-Carcinogenesis Program" (Contract FS-13, AEC-NCI, Oak Ridge National Laboratory) a number of long-term studies are underway, to determine the effects of chemical and biological agents on the respiratory tract tumor response to known chemical carcinogens. The effects of the inhalation of synthetic smog and ferric oxide particulates on lung carcinogenesis by diethylnitrosamine are being evaluated. The effects of nitrogen dioxide inhalation on respiratory tissues of hamsters and rats, are being studied. Rats and hamsters exposed to nitrogen dioxide show definite epithelial hyperplasia in bronchi, bronchioles, and alveolar ducts. The role of infections in respiratory tract carcinogenesis is also being evaluated; respiratory infection markedly enhances the incidence of lung tumors in rats exposed to N-nitrosoheptamethyleneimine, as well as impairing the ability of the lung to clear itself of particulate material. A new methodology has been developed that provides cytology specimens from the trachea of hamsters in the course of respiratory carcinogenesis. A striking similarity was found between the cytological changes obtained experimentally by carcinogens and those observed in human material.

In "Factors Influencing Experimental Respiratory Carcinogenesis by Alpha Radiation and Chemical Carcinogens" (Contract NIH-71-2136, Harvard University School of Public Health) interactions between benzo(a)pyrene and polonium-210 are being investigated in long-term animal studies, which are still under way. The distribution of polonium-210 in the lung has been found to be greatly modified when it is administered adsorbed onto ferric oxide particulates.

In "Studies in Pulmonary Carcinogenesis" (Contract PH43-66-962, New York University School of Medicine, Departments of Environmental Medicine and Pathology), carcinogens are administered both by surgical and inhalation techniques. Carcinoma of the lung has been induced in a number of species by surgical techniques, such as thread transfixion and intrabronchial pellet implantation. Materials whose carcinogenicity has been demonstrated have included polycyclic hydrocarbons, ionizing radiation, and compounds of chromium. Calcium chromate has been identified as one of the major agents in the chromate cancer problem. Inhalation studies supported by this contract have shown that combinations of sulfur dioxide and benzo(a)pyrene will

induce squamous cell carcinoma of the lung in the rat, although benzo(a)-pyrene alone will not. Bis-(chloromethyl)ether, a widely used industrial alkylating agent, and one to which humans have been exposed in chemical plants, has been demonstrated to be an extremely potent respiratory carcinogen at exceedingly low inhalation doses in the rat. As a result of these studies, the precautions in the industrial use of this hazardous material have been greatly increased. This contract has also shown by inhalation techniques that a dust, freshly generated by grinding polyurethane foam, is carcinogenic to the respiratory tract.

In "Studies on the Role of Vehicles and Particulates in Respiratory Carcinogenesis Bioassay" (Contract NIH-69-2148, IIT Research Institute), the effect of particle size and type of dust on lung clearance of benzo(a)pyrene has been examined. Lung retention of this carcinogen, coated on carbon particulates, was proportional to the size of the particle; the carcinogen was removed much more rapidly from the lung when coated on small (0.5-1.0 micron), rather than larger (greater than 2 micron) carbon particles. The rate of benzpyrene clearance from the lung was much faster when this carcinogen was adsorbed to ferric oxide or alumina particles, as compared to carbon particles. All three particulates inhibit lung clearance of the carcinogen, when rates of clearance are compared to experiments in which benzpyrene is administered without particulate matter. In this project, scanning electron microscopic studies of tracheobronchial epithelium (before and after administration of carcinogens) have just begun.

In "Studies on the Role of Vehicles and Particulates in Respiratory Carcinogenesis Bioassay" (Contract NIH-69-2144, College of Veterinary Medicine, Ohio State University), long-term animal studies are underway to determine the role of Tween 80 and other vehicles in benzpyrene carcinogenesis of the respiratory tract. The carcinogenicity of benzpyrene, adsorbed on carbon or alumina particles, rather than iron oxide particles, is also being determined in long-term studies. It is still too early to give a definitive report of these experiments.

In "Carcinogenesis Research and Bioassays" (Contract PH43-68-959, Eppley Institute for Research in Cancer, University of Nebraska), the lung cancer program has continued with studies on intratracheal administration of carcinogens to hamsters. The polynuclear hydrocarbon, 7H-Dibenzo(c,g)carbazole, which is found in tobacco smoke, has been shown to be an extremely potent respiratory carcinogen. Because of the additive effect of ferric oxide when given in combination with carcinogens, other dusts are being studied in hamsters, both alone, and in combination with diethylnitrosamine (DEN). A significant finding has been that the concomitant administration of arsenic oxide dust (along with DEN) has greatly increased the incidence of tracheal tumors, in comparison to the control group which received DEN alone.

In "Investigation of the Co-Carcinogenicity of Specific Industrial Pollutants and Cigarette Smoke" (Contract PH43-68-1372, Battelle Memorial Institute, Pacific Northwest Laboratories), the co-carcinogenicity of chrysotile asbestos, cobalt oxide, and nickel oxide, in conjunction with inhalation of cigarette smoke, is being investigated in hamsters. Aerosols of these materials have been given to hamsters in special inhalation chambers, for

prolonged periods of time. The animals are now being held for long-term final evaluation, which will be done during the coming year.

In the contract on "Role of Vitamin A in the Control of Differentiation and Carcinogenesis in the Respiratory Tract" (Contract NIH-69-2083, Massachusetts Institute of Technology), the inhibitory effects of vitamin A ester (retinyl palmitate) and vitamin A acid (retinoic acid) on benzpyrene respiratory carcinogenesis in the hamster are being evaluated. The animals are being maintained for their entire lifetime on special synthetic diets, to enable accurate measurement of total vitamin A intake in both experimental and control groups. Again, these are long-term experiments whose final results will not be available for some time.

CONTRACT NARRATIVES

BATTELLE-NORTHWEST LABS., INC. (NIH-NCI-E-68-1372

Title: Inhalation Co-Carcinogenicity of Industrial Pollutants and Cigarette Smoke

Contractor's Project Director: Dr. Alfred Wehner

Project Officer (NCI): Mr. Raymond Newell

Objectives: The project is devoted to a long-term inhalation bioassay of combined exposures to cigarette smoke and a dust (chrysotile asbestos, cobaltous oxide or nickelous oxide). Syrian golden hamsters are exposed to fresh cigarette smoke in a Hamburg-type 2 machine and to whole body dust exposure alternating with the smoke exposures. The dust distribution and characterization within the chambers are being obtained. Analysis of inhaled chemicals in tissues is performed at intervals by suitable techniques (atomic absorption spectrophotometry, neutron activation, etc.). Detailed pathological studies are conducted in all animals.

Major Findings: The long-term tests are under way. An improved asbestos disseminator has been developed. Tests have been conducted to determine the lung retention of the dusts after inhalation exposures and the translocation of the three materials from the gastrointestinal tract to the liver and kidney.

Significance to Biomedical Research and the Program of the Institute: This project is the only one in the Carcinogenesis Program designed to test the combined effects of exposures to cigarette smoke and environmental dusts. The three dusts were selected on the basis of previous studies where direct administration of the single materials in the respiratory tract was employed.

Proposed Course: The experiment is now about 18 months into its chronic exposure phase. Exposures will continue into the fall of 1972. Final evaluation of the effects and compilation of the data will require about six months so that the entire project will take approximately one more year.

Date Contract Initiated: June 28, 1968

Current Annual Level: $181,050

UNIVERSITY OF CALIFORNIA (NIH-NCI-E-70-2218)

Title: Correlation of Effects of Chemical Carcinogens in Human, Primate, and Hamster Respiratory Epithelium *in vitro*

Contractor's Project Director: Dr. T. Timothy Crocker

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: The aim of this project is to answer two questions: 1) Do specific chemical carcinogens (which are known to cause bronchogenic carcinoma in animals) cause the same sort of preneoplastic changes in the normal respiratory epithelium of man that they are known to cause in the respiratory epithelium of animals? 2) Can the anti-carcinogenic effect of vitamin A on respiratory epithelium, which has been demonstrated in hamsters (in long-term experiments *in vivo* and in organ culture *in vitro*), be meaningfully reproduced and defined in the respiratory epithelium of rodents and primates, as well as of man?

The specific experimental tool which this project uses to answer both these questions is the tracheobronchial organ culture system which Crocker and his colleagues have developed over the past six years. They are trying to find specific answers to the following questions: 1) Do respiratory carcinogens such as benzpyrene and other polynuclear hydrocarbons, nitrosomethylurethane, or others, when applied to explants of human trachea or bronchus, cause the same development of squamous metaplasia, cellular pleomorphism, atypia, or other pre-neoplastic changes, that they cause when applied to explants of trachea or bronchus from rodents or primates? 2) Does the administration of vitamin A to organ cultures of tracheobronchial epithelium, either before, during, or after the administration of chemical carcinogens such as benzpyrene nitrosomethylurethane or nitrosamines, block the development of pre-neoplastic changes caused by these carcinogens? Is this effect similar in animals as in man?

Major Findings: The contractor has shown that vitamin A blocks the neoplastic effect of benzpyrene on isolated hamster tracheal epithelium. In addition, he has shown that vitamin A does not alter microsomal metabolism of benzpyrene, suggesting that the effect of vitamin A may be a more fundamental one on differentiation. Very recently the contractor has also found that vitamin A blocks the neoplastic effects of diethylnitrosamine (DEN) on isolated hamster tracheal epithelium. Although it has been difficult for the contractor to obtain enough human tracheal material for complete studies, correlations are beginning to appear between human and animal studies.

In addition, this contract has recently demonstrated that the lesions of vitamin A deficiency in the hamster trachea can be reversed by administration

of vitamin A to organ cultures of the trachea *in vitro*.

Significance to Biomedical Research and the Program of the Institute: It is not established that polycyclic aromatic hydrocarbons such as benzpyrene can cause bronchogenic epidermoid carcinoma in rodents (such as hamsters). The contractor has recently observed the same effect in a prosimian primate, the bushbaby, *Galago crassicaudatus*. Moreover, epidemiological evidence strongly incriminates polycyclic aromatic hydrocarbons, together with other compounds found in tobacco smoke, as etiologic agents for bronchigenic carcinoma in man.

The tracheo-bronchial organ culture system developed by Dr. Crocker offers an excellent opportunity to study the direct effects of such chemical carcinogens on respiratory epithelium, and a unique opportunity to correlate the effects of chemical carcinogens on the respiratory epithelium of both animals and man. Thus, tracheobronchial organ culture is currently the only available experimental system in which one can investigate the following question: Do specific chemical carcinogens cause the same type of pre-neoplastic changes in the normal respiratory epithelium of man that they are known to cause in the respiratory epithelium of experimental animals? If the answer is affirmative, then the relationship of many of the critical animal studies in the entire Carcinogenesis Program for human lung cancer will be even more important, in an immediately apparent manner. The ability to correlate studies in experimental animal pathology with human lung cancer is of utmost importance in the total program, and the current project is a direct attempt to obtain hard data in this area.

In addition, this project represents a first attempt to deal with the problem of anti-carcinogenesis using an *in vitro* test system. Although inhibition of respiratory carcinogenesis by vitamin A in the intact animal has been established, the mechanism of the phenomenon is now known. Our ability to deal with the problem of anti-carcinogenesis in the future certainly will depend to a large extent on how much we understand about its mechanism. Definition of the parameters which affect the ability of vitamin A to reverse benzpyrene or other chemical carcinogenesis should be of great future practical use in the entire Carcinogenesis Program.

Proposed Course: During the past year, the Principal Investigator, Dr. T. Timothy Crocker, moved from the University of California in San Francisco to become Professor and Chairman of the Department of Community and Environmental Medicine at the University of California at Irvine. The work has continued in San Francisco, but this is not a satisfactory situation for the long term future of this important project. Dr. Crocker does not yet have an operational organ culture research laboratory in Irvine. It will be necessary to terminate this contract in San Francisco, and to evaluate the possibility of initiating a new contract at Irvine if adequate facilities become available at that location.

Date Contract Initiated: June 22, 1971

Current Annual Level: $103,000

HARVARD UNIVERSITY SCHOOL OF PUBLIC HEALTH (NIH-NCI-E-71-2136)

Title: Factors Influencing Experimental Respiratory Carcinogenesis by Alpha Radiation and Chemical Carcinogens

Contractor's Project Director: Dr. John Little

Project Officer (NCI): Dr. Norbert Page

Objectives: To determine whether a synergistic or potentiating effect exists between alpha radiation and chemical carcinogens in the production of lung cancer. Polonium-210 will be used as a local source of alpha radiation, benzo(a)pyrene (BP) as a local chemical carcinogen and diethylnitrosamine (DEN) as a systemic carcinogen.

Major Findings: In an earlier study by Dr. Little, lung cancer was induced in hamsters by the intratracheal instillation of either polonium-210 or benzo(a)pyrene (BP) adsorbed onto hematite particles. No tumors were found in the untreated or hematite-treated controls. Preliminary distribution studies with polonium either in saline or in various concentrations of hematite particles indicate that the uniform distribution of alpha-emitting radionuclides as occurs in saline instillation is more carcinogenic than non-uniform distribution or "hot spots" as seen with the particulate instillation.

Significance to Biomedical Research and the Program of the Institute: The proposed studies are addressed to a very critical problem in the evaluation of human lung cancer hazards which may result from the combined effects of radiation and chemical carcinogens. Synergistic relationships between alpha radiation and chemical carcinogens have been implicated in the high incidence of occupational lung cancer in man (uranium, hematite, and flurospar miners). Previous studies by Dr. Little and his colleagues have shown that polonium-210, a component of tobacco smoke, accumulates in the bronchial wall, especially the segmental bifurcations of smokers. This is a region where a high percentage of the bronchial carcinomas are found. The possible role of polonium-210, acting as a cofactor along with other chemicals in tobacco smoke, needs clarification. Results from this project could have a direct bearing on the evaluation of lung cancer hazards in man, and on future developments in the whole Special Lung Cancer Program.

Proposed Course: It is expected that these studies will require another three years to complete. Specific experiments now underway are to (1) determine the role of hematite carrier particles in radiation carcinogenesis, and (2) identify doses of BP and polonium-210 which given individually will result in a small but measurable tumor incidence. Future studies will be to (1) administer both BP and polonium-210 simultaneously and sequentially to determine synergistic action and, (2) administer DEN systemically following treatment with either BP or polonium-210 to determine possible synergism of local and systemic carcinogens.

Date Contract Initiated: May 30, 1971

Current Annual Level: $79,614

IIT RESEARCH INSTITUTE (NIH-NCI-E-69-2148)

Title: Role of Vehicles and Particulates in Respiratory Carcinogenesis Bioassays

Contractor's Project Director: Dr. Mary Henry

Project Officer (NCI): Dr. David Kaufman

Objectives: To determine the effect of intratracheal instillation of particulates of varied chemical and physical properties and of various types of solvents on the induction of epidermoid carcinoma of the lung by chemical carcinogens in the hamster.

Major Findings: Chemically and physically defined particulates have been produced by another contract at this institution (NIH-NCI-E-70-2245). Long-term carcinogenesis studies are now in progress to determine the effect of these particles of different size and composition on the induction of lung tumors by diethylnitrosamine (DEN). The experimental results available to date indicate that the particle size does not have a marked influence on tumor incidence or location of tumors in the respiratory tract. The vehicle used in this study, gelatin-saline solution, may have an effect on carcinogenesis produced by DEN, particularly in the peripheral lung. Studies on vehicles for carcinogen administration have shown that benzo(a)pyrene (BP) suspended in a gelatin-saline solution can produce lung tumors, when large amounts of the carcinogen are administered at each inoculation. Hamsters which received only the vehicle did not develop central or peripheral lung tumors. Smaller amounts of BP can also induce lung tumors in hamsters when the carcinogen is given with iron oxide dust. The physical characteristics of the carcinogen-dust combination may have a marked influence on tumor induction. Preliminary results of a study where three suspensions were prepared by different methods, indicate that the carcinogen must be in close association with the dust particle in order to produce a high tumor incidence. Another parameter being investigated is the effect of mode of anesthesia or the anesthetic agent employed on the carcinogenicity of BP-hematite. Short-term studies in progress are studying the influence of various particles on the rate of disappearance of benzo(a)pyrene following intratracheal instillation. Both the particle size and physical characteristic of the dust have an effect on the residence time of the carcinogen in lung. Dusts, such as carbon, which have a large surface area reduce the lung clearance of the carcinogen to a greater extent than the more dense materials, e.g. iron oxide. Supplemental effects have been directed toward determining the effects of hematite alone and in combination with benzo(a)pyrene on the morphology of respiratory epithelial surfaces as viewed by scanning electron microscopy. Hematite particles have a limited effect on surface epithelium, causing a roughing of the surface of non-ciliated epithelial cells in the trachea. When particles coated with the carcinogen are present in the respiratory tract, the non-ciliated cells appear irregular and misshapen, and there is a marked loss of cilia throughout the trachea and main stem bronchi. Some weeks after the end of the treatment, the entire surface is covered by large flattened cells and is almost devoid of cilia.

Significance to Biomedical Research and the Program of the Institute: This project is one of several designed to develop techniques for studying the most common type of lung cancer found in man. It is based on previous observations that bronchogenic epidermoid carcinomas could be induced in hamsters by the intratracheal administration of benzo(a)pyrene with iron oxide dust and that lung tumors can be induced by subcutaneous injection of diethylnitrosamine followed by intratracheal instillation of dusts. Further elaboration of these observations is needed to determine the role of atmospheric dusts or cigarette smoke particulates on the development of bronchogenic carcinoma in man.

Proposed Course: The project will continue with long-term carcinogenesis studies designed to determine the essential characteristics of particles as they affect the induction of epidermoid carcinoma of the lung. These studies will include investigations into the relationship between physical characteristics of carcinogen-coated particles and their role in tumor induction. Supplemental efforts in morphology of respiratory epithelial surfaces as viewed by scanning electron microscopy will continue. These studies will include investigations into the effect of particulates and carcinogens on the morphology of the respiratory tract as well as the effects of other irritants and vitamin A on morphologic changes and tumor induction.

Date Contract Initiated: June 27, 1969

Current Annual Level: $297,000

MEDICAL COLLEGE OF WISCONSIN (NIH-NCI-E-69-2082)

Title: Autoradiographic Study of the Cellular Response of the Respiratory Tract in Chemical Carcinogenesis

Contractor's Project Director: Dr. Hollis Boren

Project Officer (NCI): Dr. Curtis Harris

Objectives: The objective of this project is to determine the proliferative cellular response of various segments of the respiratory tract following exposure to respiratory carcinogens and particulates, using primarily the hamster intratracheal instillation model which is studied in other respects in several other contracts. These measurements are correlated with the histopathological response to carcinogens and particulates. In addition, radioactive carcinogens and related compounds will be localized in the respiratory epithelium by quantitative light microscopic autoradiography.

Major Findings: Work accomplished in the first and second year demonstrated specific time peaks for the cellular response to intratracheal instillation of benzo(a)pyrene, ferric oxide, or their mixture in single or repeated doses and with different sequence. A number of carcinogens, particulates and solvents were compared. Dibenz(a,h)anthracene and methylcholanthrene caused a smaller increase in DNA synthesis than benzo(a)pyrene. The carrier dust, iron oxide, produced a significant increase in DNA synthesis, whereas titanium

dioxide caused no response. Other contracts are evaluating the co-carcinogenicity of carrier particulates including titanium dioxide. The intratracheal instillation of flurocarbon solvent, FX80, produced a marked increase in DNA synthesis. An increased incorporation of tritiated uridine preceded the peak of DNA synthesis by 12 hours.

There have been two principal findings from these studies. First, the number of major cell types in tracheal epithelium is dependent upon the itamin A state of the animal. Vitamin A deficiency increases the number of basal cells and diminishes the number of ciliated cells. Hypervitaminosis A is associated with a marked increase of ciliated cells.

The second major result of this contract has been the development of a method which allows quantitative autoradiography of tracheal epithelium for the first time. With this method not only can the sequence and localization of changes be identified but valid comparisons can be made between animal experiments performed differently by different investigators. When used in this way, autoradiography becomes a bridge between morphologic and biochemical quantitative studies to determine the cellular and intracellular binding sites of ^{3}H-benzo-(a)pyrene and ^{3}H-retinoic acid which are in progress.

Significance to Biomedical Research and the Program of the Institute: This contract is an integral part of an interlocking group of contracts designed to define the role of carcinogens and the physico-chemical factors (e.g., particulates) required for respiratory carcinogenesis. The other studies, including long-term tests, have been designed so that they are closely related to the present one with maximum interaction but no overlap of activity, in an effort to define the key role of vehicles and particulates in the process of respiratory carcinogenesis.

Proposed Course: Quantitative methods have been developed so that turnover of the various cell types in the respiratory epithelium can be accurately determined following carcinogen exposure or exposure to possible promoting agents. The effect of the induction or inhibition of aryl hydroxylase(s) on the binding of ^{3}H-benzo(a)pyrene to respiratory epithelial cells will be determined. In addition, preneoplastic lesions induced by benzo(a)pyrene will be studied by high-resolution light microscopy and autoradiography.

Date Contract Initiated: June 2, 1971

Current Annual Level: $84,000

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NIH-NCI-E-69-2083)

Title: Role of Vitamin A in the Control of Differentiation and Carcinogenesis in the Respiratory Tract

Contractor's Project Directors: Dr. George Wolf, Dr. Paul Newberne

Project Officer (NCI): Dr. Michael Sporn

Objectives: It has been observed that excess vitamin A inhibits not only squamous metaplasia, but also the development of squamous cell carcinomas from the tracheobronchial mucosa in benzpyrene treated hamsters. (Saffiotti et al. 1967)

The specific aims of this Project are the following: 1) To investigate the properties, biochemical and histological, of the trachea and lung with respect to glycoprotein secretion and their response to excess and deficiency of vitamin A and vitamin A acid; and 2) To investigate the biochemical and histological response of respiratory epithelium to benzpyrene carcinogenesis with either retinol or retinoic acid treatment, at different dosages and times.

Major Findings: In an investigation of the antagonism between a carcinogen and vitamin A in respiratory tract, lung and tracheal glycopeptides from hamsters have been isolated after incubating the tissues with labeled sugar precursors. Several glycopeptides were isolated from lung tissue, and none were affected by either deficiency or excess of vitamin A. On the other hand, trachea responded to vitamin A deficiency by almost complete disappearance of a glycopeptide elutable with 0.2 N LiCl from DEAE-Sephadex. In normal trachea, this glycopeptide was virtually the only one containing fucose. It did not respond to excess retinyl palmitate feeding, but its labeled fucose uptake doubled when the animals were fed excess retinoic acid for 6 weeks. It was further purified and shown to have a molecular weight of about 5000 daltons and also to contain xylose, mannose, galactose, glucose, and hexosamines in the ratio 2.1:2:4:3:1.1, as well as sulfate and fucose.

Biochemical work is in progress on a more rapid isolation of the vitamin A-responsive glycoprotein from trachea as distinct from the glycopeptide isolated until now.

Significance to Biomedical Research and the Program of the Institute: A complete understanding of the phenomenon whereby vitamin A inhibits respiratory carcinogenesis is one of the most important goals of the entire Lung Cancer Program. This is of great significance for the problem of lung cancer in man, as well as for the problem of inhibition of carcinogenesis in the experimental animal. In other aspects of the program, plans are being made for pilot studies to see if vitamin A or vitamin A analogs can lessen cancer incidence in human populations at high risk for the development of lung cancer. The optimal outcome of these studies will depend on how fully we can develop good animal models for the intelligent use of vitamin A or vitamin A analogs as anti-carcinogenic agents. These studies in turn will depend on a better understanding of the cellular and biochemical processes controlled by vitamin A. The present contract is immediately concerned with obtaining further information in the above areas.

Proposed Course: To continue the long-term animal studies already begun, so that complete evaluation of the gross and microscopic pathology can be made. To continue with biochemical studies on the role of vitamin A in controlling normal differentiation of respiratory epithelium.

Date Contract Initiated: June 18, 1969

Current Annual Level: $200,282

UNIVERSITY OF NEBRASKA (EPPLEY INSTITUTE FOR RESEARCH ON CANCER) (NIH-NCI-E-68-959)

Title: A Resource for Carcinogenesis Bioassays and Related Research

Contractor's Project Director: Dr. Philippe Shubik

Project Officer (NCI): Dr. Gio Gori

Contract narrative is reported under the Summary Report for the Office of the Associate Scientific Director for Carcinogenesis.

NEW YORK UNIVERSITY (NIH-NCI-E-66-962)

Title: Pulmonary Carcinogenesis

Contractor's Project Director: Dr. Marvin Kuschner

Project Officer (NCI): Dr. Richard R. Bates

Objectives: The conditions required for the induction of bronchogenic carcinomas and other pulmonary tumors are tested in a series of experiments using administration of polynuclear hydrocarbons (methylcholanthrene and benzo(a)pyrene) to rats and hamsters by the following techniques: 1) intrabronchial pellet implantation; 2) intratracheal instillation of fine suspensions; and 3) inhalation exposure to finely dispersed particles in specially designed chambers.

Using these biological models, additional problems studied include a) determination of the synergistic effects of sulfur dioxide and nitrogen dioxide inhalation with benzpyrene carcinogenesis; b) Halo-ethers - Inhalation tests with chloromethyl ether and bis(chloromethyl)ether will be completed; c) Chromates - Intrabronchial pellets filled with calcium chromate have induced bronchogenic squamous cell carcinoma at the site of implantation. Inhalation tests are now underway; d) Fiberglass - Intratracheal instillation tests of fiberglass-reinforced plastics have so far proven negative for tumorigenic effects. A new apparatus has been developed for the production of atmospheric dispersions of fiberglass-plastic particles and pilot inhalation tests have been conducted; e) Polyurethane - Similar exposures are used for polyurethane plastics; f) All these tests involve supporting studies of particle size, dispersion techniques, monitoring of inhalation exposures and analytical techniques; g) In addition to pathological study of the test animals, tissue cultures of pulmonary macrophages (up to seven days) are used to test toxicity and phagocytic activity; h) a series of tests has been set up using intrabronchial pellet implantation of tobacco smoke condensate in rats, with or without addition of benzo(a)pyrene.

Major Findings: Long-term experiments are continuing in two major areas, the investigation of combinations of chemicals in the induction of respiratory tumors in rodents and the investigation of environmental materials which are

suspected of being hazardous for man. In the first category, experiments are underway to confirm the previous observation that combined inhalation of polycyclic-hydrocarbons and sulfur-dioxide acts synergistically in inducing lung tumors. In addition, similar experiments are underway with inhalation of polycyclic hydrocarbons combined with nitrogen-dioxide. These experiments have not reached the point at which conclusions can be reached. These experiments in rats are being complimented by experiments in hamsters in which polycyclic hydrocarbons are inoculated into the respiratory tract and then the animals are exposed to sulfur-dioxide by inhalation.

Preliminary investigations are underway to develop a model for the study of promoting activity in the respiratory tract. Inhalation procedures and doses for croton oil exposure have been investigated and the batch of croton to be used has been checked in a skin experiment in mice to confirm its activity.

In the second category, the investigation of materials suspected of being hazardous in man, experiments are continuing on the testing of polyester fiberglass plant dust and reinforced polyurethane foam. Inhalation of polyurethane foam insulation material for thirty days led to the development of centrolobular emphysema and several lung tumors in rats. This observation has been of considerable concern to the industries manufacturing and using these materials. They are now cooperating with the contractor to supply polyurethanes of known chemical and physical composition which can be tested in future experiments. These should lead to more definitive experiments than the previous ones done with materials of unknown composition.

Inhalation experiments are being continued with alpha Halo-ethers and with chromates. A major experiment on the effect of tracheal implants of tobacco tars is still underway.

Significance to Biomedical Research and the Program of the Institute: This contract represents a component of a broad effort to develop bioassay techniques for studies on lung cancer; it is also important for its study of potential hazards of materials that are in use industrially and may be found in the environment.

Proposed Course: The present projects of this contract are under terminal support because of the Principal Investigator's change of institution. It is proposed to continue support until all experimental work is completed and adequately reported.

Date Contract Initiated: June 29, 1966

Current Annual Level: $48,688

OHIO STATE UNIVERSITY (NIH-NCI-E-69-2144)

Title: Study of the Role of Vehicles and Particulates in Respiratory Carcinogenesis Bioassay

Contractor's Project Director: Dr. Robert Farrell

Project Officer: Dr. David G. Kaufman

Objectives: To determine effect of intratracheal instillation of fine particulates of varied chemical and physical properties and of various types of solvents on the induction of carcinoma of the lung by chemical carcinogens.

Major Findings: A long-term carcinogenesis experiment to examine the effects of Tween 80 given together with benzo(a)pyrene (BP) via intratracheal instillation in a gelatin-saline vehicle, is now nearing completion. Preliminary results suggest that animals treated with Tween 80 in addition to BP may develop slightly fewer malignant respiratory tract tumors than do those animals treated with BP alone. For another group of experiments, chemically and physically defined fine particles, both coated with BP, and uncoated, have been produced by another contract (IIT Research Institute - NIH-NCI-E-70-2245). Long-term carcinogenesis studies are now in progress to determine and compare the carcinogenicity of these preparations. In addition, long-term carcinogenesis studies have been initiated within the past year to determine the effect of uncoated particles of different size and composition, administered via intratracheal instillation, on the induction of lung tumors by diethylnitrosamine (DEN).

Significance to Biomedical Research and the Program of the Institute: This project is one of several designed to develop techniques for studying the most common type of lung cancer found in man. It is based on previous observations that epidermoid carcinomas of the lung could be induced in hamsters by the intratracheal administration of iron oxide dusts along with the intratracheal administration of benzo(a)pyrene. Further elaboration of these observations is needed to determine the role of atmospheric dusts or cigarette smoke particulates on the development of bronchogenic carcinoma in man.

Proposed Course: During the present contract year, those experiments which examine combined effects of BP and Tween 80 should be completed. Additional years will be required to complete long-term carcinogenesis studies designed to determine the following factors: a) the essential characteristics of particles coated with BP, and uncoated, as they affect the induction of epidermoid carcinoma of the lung, and b) the enhancement by chemically and physically defined particles, of respiratory carcinogenesis by diethylnitrosamine.

Date Contract Initiated: June 27, 1969

Current Annual Level: $117,733

AEC-NCI INTERAGENCY AGREEMENT, OAK RIDGE NATIONAL LABORATORY (NIH-NCI-FS-64-13)

Title: NCI-AEC Carcinogenesis Program - Research on Co-Carcinogenesis

Contractor's Project Director: Dr. James Liverman

Project Officer (NCI): Dr. Allen Heim

Contract narrative is reported under the Summary Report for the Office of the Associate Scientific Director for Carcinogenesis.

SOUTHERN RESEARCH (NIH-NCI-E-72-2064)

Title: Organ Culture Assay of Vitamin A Analogs

Contractor's Project Directors: Dr. Lee Wilkoff, Dr. Donald Hill

Project Officer: Dr. Michael B. Sporn

Objectives: This is a new contract, which began in December 1971. In other experimental work, vitamin A has been shown to have an anti-carcinogenic effect in several different test systems involving whole animals. The utility of vitamin A as an anti-carcinogenic agent is limited by undesirable toxic side-effects which result from high doses of this material. The Carcinogenesis Program, in other activities, is currently planning the procurement of new analogs of vitamin A, which are intended to have a higher degree of selectivity, that is, a higher ratio of anti-carcinogenic effects with respect to undesirable toxic side-effects. The objectives of the present contract are 1) to develop adequate bioassays to evaluate the biological activity of these new analogs (as measured by their control of epithelial differentiation) and 2) to perform bioassays on selected analogs.

At present, there are no defined quantitative bioassays for vitamin A activity in organ culture or tissue culture. Consequently, the first objective of this contract will be to develop new assays for vitamin A activity on a quantitative basis.

Major Findings: The test systems that are being employed in this contract are the following: 1) Chick Embryo Skin in organ culture. In this system, vitamin A induces mucus metaplasia, which can be scored on a morphological, histochemical, and biochemical basis; 2) Mouse Prostate in organ culture. In this system, absence of vitamin A in the culture medium leads to squamous metaplasia of the normal glandular epithelium, and the presence of vitamin A maintains normal differentiation. This system can be scored with both morphological and biochemical endpoints; 3) Hamster Trachea in organ culture. In this system vitamin A can prevent the development of pre-neoplastic lesions caused by respiratory carcinogens such as benzpyrene. The system can be scored with both morphological and histochemical endpoints. All three of the above systems are being standardized with retinyl acetate as the vitamin A reference substance. Inasmuch as this contract has just begun in December 1971, it is still too early to report any definitive results, as of March 1972.

Significance to Biomedical Research and the Program of the Institute: Vitamin A is definitely known to be required for the normal differentiation and growth of the epithelium in the following organs, all of which are major sites of primary cancer in man: bronchi and trachea, colon, stomach, uterus, kidney and bladder, testis, and skin. The role of vitamin A in controlling

normal differentiation and growth in tracheo-bronchial and intestinal epithelium is currently under intensive investigation in the Lung Cancer Unit, Office of the Associate Scientific Director for Carcinogenesis.

In addition to controlling the differentiation of many types of normal epithelium, vitamin A is also able to alter the process of chemical carcinogenesis in several different epithelia, and to block the effects of several different carcinogens. Thus, it has been found that vitamin A can block the carcinogenic effects of polycyclic hydrocarbons on tracheo-bronchial, gastric, and uterine (cervical) epithelium. In some of these studies it has been shown that the effect of vitamin A is not the trivial one of altering the immediate metabolism of the carcinogen, since vitamin A was able to inhibit carcinogenesis even when administered several weeks after the carcinogen. Studies are currently in progress in the Lung Cancer Unit, Office of the Associate Scientific Director for Carcinogenesis, which are attempting to define the biochemical and cellular role of vitamin A in blocking epithelial carcinogenesis. Presumably, this effect is directly related to the biochemical and cellular role of vitamin A in determining normal differentiation in these tissues, but the proof of this is not yet at hand.

The ultimate implications of these studies on the inhibition of epithelial carcinogenesis are very important. If vitamin A is indeed functioning as a differentiation hormone in this situation, then it should be possible, with the skill and cooperation of a group of organic chemists, to design and synthesize new analogs of vitamin A which will have even greater potency than vitamin A itself on differentiation of epithelium. The precedents in steroid chemistry for this approach to vitamin A are very impressive; presently there are many synthetic steroid analogs which are much more potent (and often more specific and less toxic in action) than the naturally occurring estrogens, progestational agents, androgens, or adrenocortical steroids. If this type of approach could be applied to the hormonal action of vitamin A, then the potential for inhibiting carcinogenesis in many types of epithelia would be greatly increased.

Proposed Course: To continue with this newly developed program.

Date Contract Initiated: December 10, 1971

Current Annual Level: $165,000

SUMMARY REPORT

TOBACCO RESEARCH SEGMENT

Fiscal Year 1972

The Tobacco and Smoking Products Research studies are continuing with the expert advice of the Tobacco Working Group, under the chairmanship of the Assoc. Scientific Director for Program, Etiology. The task of this group is to advise on the program aimed at the development of a less hazardous cigarette. The activity within the tobacco segment is articulated in the following phases: selection, preparation, and characterization of different types of tobacco and tobacco smoke condensates for subsequent bioassay and analysis; bioassay of tobacco smoke and its fractions in a battery of animal systems; chemical analysis of the tobacco smoke and its fractions; the evaluation of the data obtained in relation to the human situation.

The bioassay studies are designed to utilize different types of tobacco smoke and condensates in bioassay with existing animal systems, with particular emphasis on topical carcinogenicity by mouse skin application of condensates, and for a cytotoxic and ciliary inhibitory effect on in vitro and in vivo systems. Bioassays of 21 types of cigarette smoke condensates have just been completed in mouse skin painting tests. The data from these studies are currently being subjected to statistical analysis. The results from these studies should be available within the next two to three months, however, preliminary evaluations show that all 21 types of smoke condensates caused tumor induction under the conditions of the experiment.

A second series of experiments has been initiated utilizing 25 different types of experimental cigarettes. Emphasis has been given to low nicotine and high and low nitrogen fertilization as well as expanded types of tobacco in these studies. The protocol used in previous mouse skin painting experiments will be followed exactly so that correlation of results can be made.

Feeding tests and intratracheal instillation tests are well under way and results from these studies will be correlated with interlocking collaboration of several contractors. A series of inhalation tests using one type of cigarette are nearing conclusion and their outcome will be evaluated for determining the feasibility of establishing large scale inhalation programs.

Improved inhalation devices are being developed and tested with the collaboration of NIH staff and the AEC-Oak Ridge National Laboratories. Studies to date indicate that definite improvement can be made in the existing apparatus for smoke inhalation studies on small animals. Along with the inhalation chamber development, a concerted effort is being applied toward development of a cannulation device for bypass of the nose and pharynx in the small experimental animal.

The above studies, supported by the special appropriation for the Lung Cancer Task Force, are closely correlated to the studies on respiratory carcinogenesis conducted in the carcinogenesis program.

The major accomplishments obtained in Fiscal Year 1972 in the Tobacco Segment are summarized as follows.

Tobacco smoke condensates have been produced from 21 different types of cigarettes by mechanical smoking machines utilizing approximately 5 million experimental prepared cigarettes. The condensates have demonstrated tumor inducing activity in mouse skin painting experiments. Data obtained from this work are undergoing statistical analysis at the present time.

Studies on cilia toxicity in vivo and in vitro are presently being conducted on the 21 different tobacco smoke condensates.

Dimethylnitrosamine has been identified in specific tobacco smoke condensates. Further studies are underway to determine if this compound is present in condensates at the time of smoking or is formed as a result of chemical reacti during condensate storage.

Chemical analysis of all tobacco smoke and tobacco smoke condensates are bein performed. These results will be correlated with results from bioassay syste

CONTRACT NARRATIVES

AMERICAN HEALTH FOUNDATION (NIH-NCI-E-70-2087)

Title: Evaluation of Carcinogenic Agents in Cigarette Smoke; Biological and Chemical Assays and Epidemiological Studies

Contractor's Project Directors: Dr. Ernest L. Wynder, Dr. Dietrich Hoffmann

Project Officer (NCI): Dr. Gio B. Gori

Objectives: Investigation of the relationships of smoking and health. Laboratory investigations focus on the definition of hazards in smoke and on the development of a less hazardous cigarette. Epidemiological studies aim at identifying means of disease prevention by the definition of less hazardous smoking products and habits, and by identification of susceptible subjects and less favorable environmental conditions. A supplemental program is und way to establish the basic information on the carcinogenicity of marijuana a combined chemical and biological investigation.

Major Findings: Epidemiological studies: To date, more than 5,300 patients with tobacco-related cancers and matching controls have been interviewed in New York, Houston and Los Angeles hospitals. Preliminary analyses show a continuing trend for a reduced lung cancer risk among long-term filter cigarette smokers. For other cancers, the numbers of individuals in each cell are not large enough for statistical evaluation.

Chemical studies still in progress on tumor initiators, accelerators and promoters in cigarette smoke continue to identify more of these components in tobacco and isolate them for biological testing on mouse skin. Pyrolysis experiments are also under way. Long-term biological studies testing the carcinogenicity of condensates from high tar/nicotine and low tar/nicotine

cigarettes are still in progress.

Cigarettes made from the stems and ribs of the tobacco plant were found to have a lower tumor promoting activity than the standard tobacco, which is thought to be in part due to the greater amounts of cellulose present in the stem and rib parts of the tobacco plant. Tests on oxidized cellulose cigarettes are in progress. Carcinogenicity has also been noted for alkylated aromatic hydrocarbons found in the smoke condensate of standard cigarettes.

Chemical analyses of the physical parameters of cigarettes made from marijuana continue. No toxic effects or tumors from marijuana tar were noted during the first 14 weeks of an 18-month biological experiment. Other biological experiments with marijuana are investigating tumor promoting activity and sarcogenicity tests.

Significance to Biomedical Research and the Program of the Institute: The NCI has major responsibility in the definition of smoking hazards. Scientific expertise in this field is of vital importance in the current programs of the Lung Cancer Task Force at the NCI. Resources capable and willing to perform biochemical and epidemiological surveys in this field are extremely limited. Therefore, the outstanding position of this contract fulfills an essential need of the NCI. Additional opportunities for expansion of scientific efforts by this contractor have been identified, should the funding situation improve.

Proposed Course: To continue as outlined above.

Date Contract Initiated: February 24, 1970

Current Annual Level: $258,175

ARTHUR D. LITTLE, INC. (NIH-NCI-E-69-2147)

Title: Bioassay of the Cytotoxicity of Cigarette Smoke and of Its Effects on Ciliary Function

Contractor's Project Director: Dr. Philip S. Thayer

Project Officer (NCI): Dr. Gio B. Gori

Objectives: 1) To evaluate experimental cigarettes provided by the program for development of a less hazardous cigarette in two groups of test systems:

a. Effects of smoke on growth and phagocytic activity of *in vitro* cultivated cells.
b. Effects of smoke on ciliary kinetics and dynamics of mucus transport in the respiratory epithelium of experimental animals *in vitro* and *in vivo*.

2) To develop and evaluate a model system to determine whether cigarette smoke has effects on carcinogenesis in the chicken trachea as influenced by

experimental removal of the tracheal epithelium.

Major Findings: Two series of tests of the 23 experimental cigarettes produced for the first phase of the NCI program have been performed in all 4 bioassay systems indicated in Objective 1. Statistical analysis has shown that cigarette numbers 5, 13, 15, 17, 20 and 21 are significantly less active against cilia in vitro; 3, 7, 11, 13, 21 against cilia in vivo; 2, 3, 9 and 11 against alveolar macrophage phagocytic activity; and none of them in the cytotoxicity assay. The actual differences (although statistically significant) are small compared to those achievable by selective filtration.

Studies have been initiated to develop several parameters of the tracheal neoplasia system in the chicken. Radioautographic methods were employed to measure the mitotic activity in the upper and lower portion of the denuded trachea in an attempt to characterize cell kinetics. In general, mitotic activity appears to be greatest within the first 2 to 4 days following denudation.

A constant volume displacement smoking device has been developed for accurately smoking cigarettes and chronically dosing chickens. Additional effort is now being directed toward a technique for automating the administration of smoke to the chicken for these studies.

Neither metaplasia nor carcinoma was found in animals treated at 14-day intervals with either 2 or 4 instillations of DEN (20 mg/kg) and examined after 2.5 months.

Significance to Biomedical Research and the Program of the Institute: The overall program for the formulation of a less hazardous cigarette needs to identify physiological responses of the respiratory tract surface tissues to cigarette smoke of different characteristics.

This information will be correlated with carcinogenesis data in testing and directing modification of cigarettes towards less hazardous formulations.

Proposed Course: The four bioassay procedures are available for evaluation of subsequent sets of experimental cigarettes. The development of the neoplastic model system is proceeding.

Date Contract Initiated: June 19, 1969

Current Annual Level: $99,825 (1972)

AEC-NCI INTERAGENCY AGREEMENT (OAK RIDGE NATIONAL LABORATORIES) (NCI-FS-40-117-67)

Title: I. Collection, Separation and Elucidation of the Components of Cigarette Smoke
II. Inhalation Bioassay

Contractor's Project Directors: Dr. Michael Guerin, Dr. Wilbur Shults

Project Officer (NCI): Dr. Gio B. Gori

Objectives: I. To operate a facility for the chemical characterization of cigarette smokes, survey novel analytical methodologies for more complete chemical characterizations of smokes, and provide chemical data characterizing NCI experimental cigarettes.

II. To provide the chemical and hardware development expertise required in a joint Biology Division-Analytical Division pilot study of the utility of intratracheal cannulation in cigarette smoke inhalation bioassays.

Major Findings: I. In excess of five thousand determinations have been executed in establishing and confirming procedures and chemically characterizing the smokes of twenty-three experimental cigarettes. Parameters determined include total particulate matter, nicotine alkaloids, water, and tar contents of particulate matter; acetaldehyde, acrolein, hydrogen cyanide, carbon monoxide, carbon dioxide, formaldehyde, pH, and oxides of nitrogen contents of the gas phase; acetone, water, dry condensate, wet condensate, tar, nicotine, nicotine alkaloids, phenol, o-cresol, m + p cresol, colorimetric phenols, weak acids, very weak acids, pH, phenanthrene, benz(a)anthracene, and benzo(a)-pyrene contents of smoke condensate suspensions. The smokes of four special interest cigarettes, including marijuana, have been completely characterized. A substance thought promising for reducing the carbon monoxide delivery of cigarettes was tested as a filter material and found ineffective.

Flame photometric detection gas chromatography has been shown promising for characterizing the delivery of sulfur compounds. Spark source mass spectrometry, neutron activation analysis, and atomic absorption spectrometry have been tested for assessing the metallic contents of smoke preparations. Direct injection gas chromatography has been found promising for the rapid quantitative estimation of fatty acids in smokes.

II. An intermittent smoke generation-exposure device has been designed and fabricated which allows the simultaneous exposure of ten hamsters to alternating environments of 10% smoke and fresh air. Animal containment vessels suitable for use with cannulated animals have been produced. Novel models of mouthpiece-cannula assemblies are under development. Lung deposition methodologies using carbon-14 hexadecane and dotriacontane have been characterized.

Significance to Biomedical Research and the Program of the Institute: I. The National Cancer Institute has assumed much of the responsibility for defining the carcinogenic potential of cigarette smoking and steps which might be taken to reduce that potential. This facility provides the chemical characterization of cigarette smokes undergoing extensive biological study by other Institute contractors. The data is critical to the proper interpretation of the biological results and helpful for the design and control of future experiments. Steps taken to more completely characterize smokes and to carefully define routine analytical procedures improve the reliability, uniformity, and extent of experimental progress toward the definition of characteristics of a less hazardous cigarette.

II. The relatively poor success of attempts to induce lung tumors in experimental animals subjected to the passive inhalation of cigarette smokes has been attributed to nasal filtration and the resulting attenuated dose of smoke reaching the lungs. Providing a cannula to bypass the nose may allow sufficient smoke to reach the target organ. Production of a successful and practical inhalation bioassay will allow an examination of the carcinogenic potential of whole smokes. Both the particulate and gas phase components can contribute to the biological response. Conditions more closely approximating those of the human smoking situation can then be evaluated. The methodology can have a major impact on efforts to define the characteristics of a less hazardous cigarette.

Proposed Course: I. To continue as outlined above. To include additional parameters in the routine operations, increase efficiency through computerized data treatment and reporting, and present a finalized compilation of highly defined analytical methodologies.

II. To establish a collaborative biological-chemical program to expedite the development of a viable cigarette smoke inhalation bioassay.

Date Contract Initiated: November 1969

Current Annual Level: $200,000

MELOY LABORATORIES (NIH-NCI-E-69-2084)

Title: Preparation and Analysis of Cigarette Smoke Condensate Samples

Contractor's Project Director: Dr. A. R. Patel

Project Officer (NCI): Dr. Gio B. Gori

Objectives: Meloy Laboratories is responsible for mechanically smoking cigarettes and collection of the smoke condensates having, as nearly as possible, uniform composition for use in a number of bioassay programs to be carried out under contracts in the Chemical Carcinogenesis/Lung Cancer Task Force Program.

1) Each of the experimental types of cigarettes are conditioned at 75 $\pm$ 2°F and 60 $\pm$ 5% relative humidity for at least 48 hours prior to being smoked on two manifold type smoking machines furnished by the National Cancer Institute. The smoke condensate is collected in the glass trapping system and processed according to experimental protocol for shipment to other contractors.

At least 420 gms of dry condensate is produced from each of the different types of cigarettes every eight weeks for skin-painting study. During the past year, Meloy Laboratories has produced 50 gms of dry condensate from each type of cigarettes for the Oak Ridge National Laboratory chemical analysis studies, and 450 gms of smoke condensate from two types of cigarettes, every 8 weeks for intragastric intubation studies.

This contractor has made 1.2 million M-coded cigarettes from marijuana furnished by the NCI. About 400 grams of dry condensate is produced from these cigarettes every 8 weeks. It is hand carried to New York in a dry-ice container and delivered to Dr. Hoffman of the American Health Foundation for skin-painting study.

Beginning February 1, 1972, Meloy Labs will produce 420 gms of cigarette smoke condensate from 25 types of new cigarettes every 8 weeks for skin-painting study by the Hazleton Labs. Condensate (420 gms) will be produced from four types of cigarettes every eight weeks for skin-painting studies by the American Health Foundation.

2) The contractor conducts a quality control program of chemical analysis on all batches of cigarette smoke condensate, both to insure uniformity and to characterize the product to some extent for the subsequent users.

Major Findings: This is a complex service contract, the scope of which is production of cigarette smoke condensate on schedule, and performing quality control on each batch of smoke condensate.

Significance to Biomedical Research and the Program of the Institute: This contract is designed to produce the cigarette smoke condensate required for the execution of four other projects which are funded under the Lung Cancer Task Force: 1) Skin Carcinogenesis Bioassay of Cigarette Smoke Condensates; 2) Intratracheal Bioassay of Cigarette Smoke Condensate for Carcinogenesis; 3) Bioassay of Cigarette Smoke Condensate by Oral and Parenteral Routes; 4) Skin Carcinogenesis Bioassay of Marijuana Cigarette Smoke Condensate.

Proposed Course: Continuation of activities started in February 1970, as experimental cigarettes become available. Analytical work on the unsmoked tobacco from these experimental cigarettes will be carried out. Production activities on the new series of NCI cigarettes are proceeding on schedule.

Date Contract Initiated: June 30, 1969

Current Annual Level: $326,414

NEW YORK UNIVERSITY (NIH-NCI-E- 64-938)

Title: Studies on Carcinogenesis Principles of Processed Tobacco and Tobacco Smoke

Contractor's Project Director: Dr. Benjamin Van Duuren

Project Officer (NCI): Dr. Harry Gelboin

Objectives: The isolation and identification of tumor-promoting agents derived from tobacco smoke condensates and the determination of their contribution to the health hazards of cigarette smoking. To determine the various factors related to tobacco processing which may affect the nature of

their health hazard. This includes examining various compounds from tobacco leaf and tobacco smoke, tobacco pesticides, and development of an in vitro cell culture method for detecting co-carcinogenicity.

Major Findings: The esters decyl acetate and linalyl crotonate as well as the alcohol linalool are promoting agents on mouse skin. They have confirmed that the saturated aliphatic hydrocarbon dodecane is a promoting agent of moderate activity. This opens up once more the question of the role of aliphatic and olefinic hydrocarbons of cigarette tar as promoting or co-carcinogenic agents, since these compounds constitute a substantial proportion of the organic compounds in cigarette tar. They have developed a cell culture system for the rapid assessment of tumor-promoting activity. This system depends upon the measurement of the enhancement of transformed cell outgrowth in mixed culture (3T3 mouse fibroblasts and SV-transformed 3T3 cells). A series of compounds which are either known or suspected components of tobacco leaf and/or tar were tested in this system during the past year. A major possible benefit of this bioassay is that it may be of use in shortening the duration of a test from one year or more to a few weeks. Good agreement has been found between the in vivo and in vitro test systems. The exceptions are those in which the material on test has high toxicity. They have tested compounds for co-carcinogenic activity, i.e. by simultaneous and repeated application of the carcinogen (benzo(a)pyrene) and the compound on test for co-carcinogenic activity. This test system parallels more closely the exposure of mouse skin to tobacco tar compared to the initiation-promotion sequential application (tumor-promotion) used earlier. Some tumor-promoting agents, e.g. linalyl oleate and linalyl acetate, are also co-carcinogenic agents. The potent tumor-promoting agent phorbol myristate acetate from croton oil is also a potent co-carcinogen in this test. However, the compounds phenol, rutin and morin resulted in a decreased tumor yield and/or delayed appearance of tumors when compared to benzo(a)pyrene administered alone.

Significance to Biomedical Research and the Program of the Institute: The identification and characterization of the factors in cigarette smoke are paramount requirements in our understanding of the process of lung tumorigenesis induced by cigarette smoke. The program is clearly designed to identify these factors so that they can be either removed from the cigarette or chemoprophylaxis developed to counteract their action.

Proposed Course: To continue to examine synthetic components of tobacco tar and/or smoke, tobacco tar polyphenols and various fractions thereof, to examine the effect of the tobacco pesticide, Dasanit, on tumorigenesis and to examine the mixed cell culture system as assay system for tumor promotion. A number of new compounds will be examined for tumor promoting activity and a search will be made for alkylating agents present in tobacco smoke.

Date Contract Initiated: June 29, 1964

Current Annual Level: $98,090

TRW-HAZLETON LABORATORIES (NIH-NCI-E-71-2254)

Title: Chronic Carcinogenesis Bioassays by Intratracheal Instillation of Cigarette Smoke in Syrian Hamsters

Contractor's Project Director: Mr. James Gargus

Project Officers (NCI): Dr. John Cooper, Dr. Michael Sporn

Objectives: The objective of this contract is to evaluate the carcinogenic effects of various cigarette smoke condensates in hamsters resulting from repeated intratracheal administration.

Major Findings: Dose range studies have been completed to establish levels of administration which are suitable for the conduct of chronic studies. In addition, the long term studies have been begun during the final months of this support period.

Significance to Biomedical Research and the Program of the Institute: Work performed by Dr. Saffiotti and co-workers has already clearly established that benzpyrene and other purified hydrocarbons (which are constituents of tobacco smoke) will cause tracheal and bronchial epidermoid squamous cell carcinoma, when administered intratracheally in hamsters. The histology of these tumors closely resembles the most common form of human lung cancer. There is thus available an excellent animal model for the human disease, and the present contract will test the ability of various types of tobacco tars to induce lung cancer when administered intratracheally. These studies are an important parallel to similar work being done with purified hydrocarbons, and should ultimately represent an important bioassay for a safer type of cigarette.

Proposed Course: Following 50 weeks of repeated intratracheal instillations, the experimental animals will be observed for an additional 30 weeks. Survival and general appearance of animals will be recorded, and all animals will be weighed monthly.

Necropsy observation will be recorded at the time of death or sacrifice of the animals. Histopathological examinations will be performed on specified organs and tissues.

The cigarette smoke condensates will be supplied from NCI Contract No. NIH-NCI-E-69-2084 with Meloy Labs, Inc., Falls Church, Virginia.

Date Contract Initiated: May 20, 1971

Current Annual Level: $90,351

TRW-HAZLETON LABORATORIES (NIH-NCI-E-69-2145)

Title: Carcinogenicity Bioassays by Intragastric Intubation of Cigarette Smoke Condensates in Experimental Animals

Contractor's Project Director: Mr. James Gargus

Project Officer (NCI): Dr. John Cooper

Objectives: The objective of this activity is the evaluation of carcinogenic potential of cigarette smoke condensates following repeated oral administration in rodents.

Experimental variables in the cigarette smoke condensates will include nicotine content and total dose. Once standardization is attained, the bioassay will be used for determining the carcinogenic potential of smoke condensate from modified cigarettes, as a part of the monitoring system in the program for the development of a less hazardous cigarette.

Major Findings: During the past support period, dose range studies have been completed on an acute and subacute basis which resulted in the selection of dosage levels suitable for the conduct of chronic studies. In addition the specific strain of rat and mouse to be used in this study was selected and the long term experiments initiated.

Significance to Biomedical Research and the Program of the Institute: This project is an integral part of the program for the formulation of a less hazardous cigarette. This program requires that new bioassay techniques be devised which will be both sensitive and specific. The selection of this route of administration for emphasis is justified by the large quantity of tobacco tars and condensate which are ingested into the stomach in the course of smoking and subsequent ciliary clearance of the respiratory tract.

Proposed Course: The experimental animals will receive intragastric instillations on a continuing schedule for the remainder of their life times. Survival and general appearance of the animals will be recorded and all animals will be weighed monthly.

Necropsy information will be recorded at the time of death or sacrifice. Histopathological examinations will be performed on specified organs and tissues.

The cigarette smoke condensates will be furnished from NCI Contract No. NIH-NCI-E-69-2084.

Date Contract Initiated: June 24, 1969

Current Annual Level: $145,948

TRW-HAZLETON LABORATORIES (NIH-NCI-E-69-2149)

Title: Skin Carcinogenesis Bioassay of Cigarette Smoke Condensates in Mice

Contractor's Project Directors: Mr. James Gargus
Dr. Marcelina Powers

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The objective of this project is to provide a standardized bioassay for the determination of the carcinogenicity of cigarette smoke condensates by repeated dermal applications in mice.

Whole smoke condensates are supplied by the sponsor from Meloy Laboratories. The experimental variables included in these coded samples are tobacco of different origin; methods of cultivation, including fertilization, pesticides, suckering agents, and soils; curing and aging methods, blends of tobacco plant parts such as leaves, stalks, and stems; reconstituted tobacco, cutting of tobacco, cigarette paper, and filters.

Female ICR mice are treated six times weekly with dermal applications of the whole smoke condensates dissolved in acetone. Skin tumors are recorded and measured. All animals are necropsied and tissues are fixed and preserved for histopathological examinations. Data on each animal are entered in a computer memory once monthly. Computations and printed reports from NCI provide monitoring data for continuous evaluation of the experimental results.

Major Findings: The contract activity is summarized for the 12-month period since the last annual report was submitted on March 19, 1971. During this period, repeated treatments were made on 7300 mice through the 18-month experimental period for each group. Terminal sacrifices were made on 6,800 mice during October, November, and December 1971. The preliminary histopathological examinations on selected tissues from these mice is in progress. Dermal applications in five groups (initially 500 mice) are continuing to July 1972 when they will be sacrificed.

Plans have been completed to initiate a second series of bioassays of 25 different smoke condensates. With vehicle, positive and negative controls, 6,500 experimental animals will begin their 18-month treatment period in February, March, and April 1972.

The experiment design employed in this new series will be the same as that previously employed. Monthly animal data will be submitted for computer storage and computations.

Significance to Biomedical Research and the Program of the Institute: The objective of this project is to provide a standardized bioassay for the determination of carcinogenicity of cigarette smoke condensates by dermal application to mice. These comparative bioassay results along with other monitoring bioassays and chemical determinations will permit evaluation of the biological effects of the variables in the different smoke condensates. These comparative evaluations will provide data for testing and developing

less hazardous cigarette formulations.

Proposed Course: Initiation and performance of a second series of bioassays on 25 smoke condensates.

Date Contract Initiated: June 24, 1969.

Current Annual Level: $311,570

IV. *PUBLICATIONS OF THE CARCINOGENESIS PROGRAM*

A. *COLLABORATIVE PROGRAM*

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[1] Publications with authors from the intramural staff and from contractors' staff are reported under both lists; such cross references are provided in parentheses.

SUMMARY REPORT

OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR
FOR VIRAL ONCOLOGY (OASDVO)

July 1, 1971 - June 30, 1972

J. B. Moloney

The Annual Report for Viral Oncology, Etiology, NCI, is presented as follows:

I. Viral Oncology Area - Special Virus Cancer Program (SVCP)

- A. Introduction
- B. Organization
  - 1. Viral Oncology Area
  - 2. Special Virus Cancer Program (SVCP)
- C. Scientific Activities - Progress Highlights
- D. Projections
- E. Publications - SVCP

II. Summary Reports

- A. Offices of the Associate Scientific Director for Viral Oncology
  - 1. Office of Biohazards and Environmental Control
  - 2. Office of Program Analysis and Communications
  - 3. Office of the Coordinator for Ultrastructural Studies
  - 4. Office of Program Resources and Logistics
- B. Branch Reports
  - 1. Viral Leukemia and Lymphoma Branch
    - (a) Summary
    - (b) Individual Project Reports
  - 2. Viral Biology Branch
    - (a) Summary
    - (b) Individual Project Reports
  - 3. Viral Carcinogenesis Branch
    - (a) Summary
    - (b) Individual Project Reports

III. Special Virus Cancer Program Contract Summaries by Segments

I. VIRAL ONCOLOGY AREA - SPECIAL VIRUS CANCER PROGRAM (SVCP)

A. Introduction:

The Viral Oncology Area is responsible for planning and conducting the Institute's program of coordinated research on viruses as etiological agents of cancer. Scientists within this Area not only provide the broad operational management for intramural and collaborative research but also conduct investigations on oncogenic viruses and their interaction with host cells and apply this information to search for viruses which may be etiologically related to the initiation and continuation of human cancer.

Contract supported research is conducted within the Viral Oncology Program under the Special Virus Cancer Program (SVCP) whose primary objectives are: (1) to determine whether viruses comparable to those known to induce cancers of laboratory and domestic animals are causative agents of human cancers, and (2) to develop therapeutic and preventive measures for control of human cancers when such causative agents are found. A detailed history of events leading to the development of the SVCP may be found in previous Annual Reports of the NCI. In the eight-year history of the Special Virus Cancer Program, the National Cancer Institute has sponsored extensive research on the role of viruses as etiologic agents of cancers of man. The Program has succeeded in marshaling many of the nation's finest virologists, biochemists, immunologists, molecular biologists, epidemiologists, and physicians for this strongly goal-oriented effort. From the beginning, it was clear that an understanding of the suspected relationship between tumor viruses and human neoplasia would not only require an interaction among these groups of scientists but sound and constructive administrative support as well. Having provided the broad operational management and funds--the budget has risen from $10 million in 1964 to a projected $46 million for 1972--for an integrated contract program of international scope, Program leaders are confident that the objectives will be achieved.

The SVCP is changed, refined, and evaluated by the Science Management Team and its key advisors. The Program plan has undergone considerable revisions since its inception. This year the Research Logic Flow Chart was extensively revised and updated. It is based on the premise that a virus or viral genetic information persists in the diseased individual and is an indispensable element for the induction of certain kinds of human cancer. The Program plan is reviewed regularly by the Director, NCI; Scientific Director for Etiology, NCI; the National Cancer Advisory Board; The Scientific Directorate, NCI; and the Etiology Program Management Group, NCI.

B. Organization:

1) Viral Oncology Area. During the previous reporting period the Viral Oncology Area was reorganized into a structure that has permitted more effective use of scientists and facilities. This year one new Office was established within the Office of the Associate Scientific Director for Viral Oncology: the Office of Program Resources and Logistics. This change was effected with existing personnel, space, and funds.

This Office and its standing Advisory Committee have been given the task of implementing and coordinating contracts awarded to provide resources for both intramural and collaborative research and has assumed the responsibilities of the former Program Resources and Logistics Working Group, a Segment within the Special Virus Cancer Program. In the coming year, it is anticipated that a prime contract will be established to assist this Office in maintaining and substantially expanding the resources and integrated logistics support for the Area.

The specific functions of this Office are as follows: Plans and maintains a program to anticipate and meet the needs of the Viral Oncology Program (in house and collaborative) for resources and logistics support. Insures optimum use of government funds through careful selection of contractors, periodic review of contractors' performance, and management of collaborative research contracts. Documents all aspects of the resources and logistics program. Prepares an annual catalog which lists and describes the research resources available to the Program. Develops in collaboration with the Office of Program Analysis and Communication, a computerized central inventory for the sera, cell cultures, and human cell and tissue specimens acquired by the Program. Insures that research resources are always available for use by laboratory investigators.

Members of the Program Resources and Logistics Advisory Group are:

Dr. Jack Gruber, Chairman
Dr. Robert Bassin, NCI
Dr. James Duff, NCI
Dr. Robert Holdenried, NCI
Dr. Garrett V. Keefer, NCI
Dr. Timothy O'Connor, NCI
Dr. Wade Parks, NCI
Dr. Gary Pearson, NCI
Dr. Deward Waggoner, NCI

The Viral Oncology Area is organized as follows: The Office of the Associate Scientific Director for Viral Oncology includes four Offices: The Office of the Coordinator for Ultrastructural Studies with a Viral Studies Section; The Office of Biohazards and Environmental Control with two sections, the Biohazards Research Section and Environmental Control Section; The Office of Program Analysis and Communications with one Information Unit; and The Office of Program Resources and Logistics.

The Viral Biology Branch includes six sections: Cell Biology Section, Electron Microscopy Section, Microbiology Section, Experimental Pathology Section, Human Tumor Studies Section, and Virus and Disease Modification Section.

The Viral Leukemia and Lymphoma Branch includes the Tumor Virus Section, Immunology Section, Viral Pathology Section, Genetics Section, and Molecular Biology Section.

The Viral Carcinogenesis Branch includes the Ecology and Epizoology Section, with the Field Studies Unit; the Solid Tumor-Virus Section; and the Viral Genetics Section, with the Serology Unit, and the Trailer Unit.

2. Special Virus Cancer Program

The Special Virus Cancer Program is currently composed of 5 working segments: Developmental Research Segment, Immuno-epidemiology Segment, Biohazards Control and Containment Segment, Solid Tumor Virus Segment, and Breast Cancer Virus Segment. Since the last reporting period, three working group segments were terminated: The Immunology Group, the Special Animal Leukemia Ecology Studies Segment, and the Program Resources and Logistics Segment; and a new Segment: The Immunology-epidemiology Segment, was established.

On September 28, 1971, the Immunology Group which had served on an ad hoc basis to review and manage contract proposals of an immunological nature for the entire Etiology Area was terminated. It was expected that other review bodies in this Area would then assume these responsibilities. On September 29, 1971, at the request of the Associate Scientific Director for Viral Oncology, the Scientific Director for Etiology approved the formation of a new segment known as the Immuno-epidemiology Segment. Contracts relating to the goals of the Special Virus Cancer Program were transferred to this Segment, whose mission is as follows: To develop and improve immunological techniques relevant to the determination of the etiology of neoplasia and its detection, prevention, and treatment; to apply the information gained from these studies to human cancer, with specific reference to the detection, prevention, and treatment of human tumors, especially those of possible viral etiology.

Because the mission of the Special Animal Leukemia Ecology Studies Segment was successfully accomplished, this working group was terminated on October 1, 1971. In existence since the inception of the Program, this working group was responsible for conducting research on the etiology of animal tumors and their possible interrelationships to man. Over 100 viruses known to cause tumors in vertebrate species were discovered and characterized; evidence for a causal relationship between animal tumor viruses and human cancer was entirely negative. Contracts within this segment were distributed, therefore, to other working groups to provide for better coordination and integration of studies on model systems with research on the viral etiology of human cancer.

On March 1, 1972, the responsibilities of the Program Resources and Logistics Segment were transferred to the Office of Program Resources and Logistics in the Associate Scientific Director's office. The reasons for this change are given in Part B, item 1 of this report.

With the advent of many new immunological and biochemical methods for the detection of viral antigens and/or viral activity within cells, it is anticipated that in the next year a new segment, the Tumor Virus Detection Segment, will be established.

3. Program Management

Segment Chairmen. The program segment chairman is responsible for planning the projects in each working group. As a senior scientist, he must review, analyze and integrate studies which fulfill the objectives of his working group and the total Program. The chairman is aided in this task by a vice-chairman, executive secretary, and working group members composed of intramural and extramural scientists.

Project Officers. The project officer is the direct extension of the program segment chairman. To assure progress in accomplishing the goals set forth in the workscope of a project, he is called upon to advise the principal investigator on scientific matters and inform him of segment and program decisions.

Executive Secretaries. The executive secretary assists the segment chairman and project officers in managerial duties of contract operation. He is responsible for optimal review and documentation of each project within the working group.

Contract Specialists. The contract specialist is now responsible for negotiating research contracts. He provides valuable advice on fiscal and legal matters to the project officers, executive secretaries, segment chairmen and Associate Scientific Director. Some specialists are well conversant with the scientific aspects of the Program.

Working Groups. The program segment working group is the basic review unit for the Program. Composed of both intramural and extramural scientists and chaired by a senior NCI scientist, the group reviews individual contracts or proposals for scientific excellence and technical competence within a given funding level. Its recommendations provide the Segment Chairman, Associate Scientific Director, and the Scientific Director with a basis for program decisions.

Contract Review.

The projects within the total Program are reviewed, both formally and informally, at three levels:

(1) Each contract is reviewed for relevance, priority, and need to total Program at the Program Segment Chairmen's meeting.

(2) Each contract is reviewed for scientific excellence and technical competence at the Program Segment Working Group meeting.

(3) Each contract is reviewed for performance by the Project Officer.

As an aid to the review processes, key staff members receive progress reports on all contracts on a triannual basis. Collection and distribution of these reports is the responsibility of the Office of Program Analysis and Communications. A single comprehensive report is prepared annually by the Associate Scientific Director.

PROGRAM MANAGEMENT PERSONNEL

Science Management Team

Dr. J. B. Moloney, Associate Scientific Director for Viral Oncology, NCI
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The Special Virus Cancer Program constantly seeks the advice of its peers, to act as Program consultants. To assure the scientific excellence of its projects, at least one half of the members appointed to serve on each working group segment are non-government scientists. Additional reviews are provided by other standing advisory committees of the NCI: The National Cancer Advisory Board, and the Etiology Program Advisory Committee. These committees are composed entirely of non-government scientists.

C. Scientific Activities and Progress Highlights

The summary reports of the Office of the Associate Scientific Director for Viral Oncology and of the Offices and Branches within this Office are presented in the following sections. Highlights of these reports demonstrate that the Viral Oncology Area through its intramural and collaborative research programs has already provided a broad base of information on the characteristics of tumor viruses and is applying this knowledge to the isolation and identification of human tumor agents.

SUMMARY REPORT

Introduction. A considerable number of viruses are known which either directly or indirectly cause tumors in different vertebrate species. Many of these replicate in cultured human cells, and some cause cellular transformation. Since the RNA viruses are responsible for many naturally occurring tumors in animals, this group is most likely to be the cause of some human neoplasms. Although no virus has yet been proven to induce human cancer, it would be unreasonable to consider man to be the exception. Certain DNA viruses may also be involved in oncogenesis. Herpesviruses, in particular, are known to induce lymphoproliferative diseases in several animal species. The close association of these viruses to human malignancies suggests that they may play a causal role or act as necessary co-factors in tumor formation.

RNA VIRUSES

Characteristics. RNA tumor viruses have a number of similar biological and biochemical characteristics. They are widely distributed in vertebrate animals: the chicken, snake, mouse, rat, hamster, cat, cow, and non-human primates. Vertical rather than horizontal transmission is probably the natural means by which they are perpetuated and may account for their common occurrence in certain animals. Infections are not necessarily associated with virion release or the development of neoplasms. Apparently the information for making a Type C virus can be incorporated into the chromosomes of the host and is passed on as a heritable factor. In such covert infections, there is only partial expression of the full antigenic complement of the viral gene and the infected animal often remains tolerant to these antigens. While activation of tumor virus expression or infectious virus may occur spontaneously as a result of aging or following exposure to carcinogens in the environment, some viruses are defective and require co-infection with another virus for maturation. The host genes also have been shown to have a profound effect on the virogenic and tumorigenic expression of RNA virus in normal tissue. Immunogenetic studies on recombinant inbred lines of mice have identified the genes responsible for host response to both exogenous and endogenous virus expression, replication, and tumorigenesis and for the host range of tumor viruses. The gene responsible for gs-antigen expression (gs+) of a murine leukemia virus is transmitted as a simple Mendelian dominant; another has been identified as a

determinant of virus replication and segregates independently from the gs-determinant gene.

Each Type C virus, regardless of origin, possesses a major internal protein of approximately 30,000 molecular weight. This protein (gs-1) has a species-specific isoelectric point and carries determinant groups which permit delineation of the species of origin. The specific reactivity appears to be an invariable characteristic of the virus regardless of what cell type is used to propagate the virus. The mammalian viruses also share a determinant, perhaps on this same protein, which is inter-species specific (gs-3). A third antigen (gs-2) has been isolated recently but it is not yet well-characterized. Two group specific antigens of the Type B particles producing murine mammary tumors (s_1 and s_2) have recently been isolated and characterized. These are antigenically dissimilar to corresponding antigens of the Type C viruses. All of these viruses contain 60-70S RNA and an enzyme, RNA-dependent polymerase which has the property of assembling DNA copies of viral RNA. The enzymes from the different mammalian Type C viruses are antigenically related but not identical; those of the mouse mammary tumor virus and avian leukosis viruses are antigenically distinct. With the possible exception of certain foamy viruses and viruses producing "slow diseases", the presence of this enzyme may be unique to the RNA tumor virus group.

Information on the RNA tumor viruses together with data acquired by extensive studies on laboratory and feral mice has led to a theory of RNA viruses as determinants of cancer. It proposes that these viruses exert their oncogenic action directly on information already present in all cells, the oncogene. The information carried within the virus particle (virogene) is transmitted to succeeding generations of cells in covert form in a normal pattern of inheritance; expression is controlled by host regulator genes and repressors within the normal adult cell. Derepression, which may occur with advancing age, upon exposure to environmental carcinogens, or following infection with certain DNA viruses, results in tumor development and possibly virus release. The idea is simple: if all cells vertically inherit oncogenes, transformation to malignancy depends on whether its oncogenes are switched on; similarly, the release of an RNA tumor virus depends on whether its virogenes are also expressed.

The one major criticism of this theory--its difficulty to test--has not prevented collecting evidence in favor of it. For example, it was recently shown that apparently normal, gs+ chick cells spontaneously release a Type C virus which can be classified as an avian leukosis virus; even gs- cells, following treatment with mutagens (BrdU), and ionizing radiation, start to release a similar virus. Virus-free clones of AKR mouse embryo cells on exposure to mutagens, or following transformation with DNA viruses produce a virus identical to one that causes leukemia of AKR mice. Understanding of the mechanisms whereby the oncogene is repressed or derepressed offers excellent possibilities for preventing or modifying virus-induced cancers.

Relationship to human cancer. The nature of the virus-host relationship recognized in the RNA tumor viruses demonstrates the difficulty in establishing definitive associations of viruses to the oncogenic process in man. Several lines of investigation have been followed: human populations were studied to determine whether exposure to known viruses might be a factor in the incidence of cancer; efforts were increased to detect and recover viruses from tissues of cancer patients; and more recently, investigations were initiated to determine the molecular pathways for virus replication, integration into the host genome, and neoplastic transformation.

Studies were conducted to determine whether possession of household pets influenced the familial incidence of cancer. The ease with which the feline leukemia and sarcoma viruses infect human cells in culture suggested a potential hazard for man. Virus has been detected in the saliva and tissues of leukemic cats, and is present in some apparently normal animals. Accordingly, surveys were initiated to determine whether a significant difference existed in the incidence of cancer among families owning cats and dogs, and those without pets, but none could be demonstrated. Although no antigens reactive with an antiserum to feline leukemia virus were found in lymphomatous and non-lymphomatous tumors of other species including man, further confirmation negating natural infection of man may be required.

The recent demonstrations of three RNA viruses in different primate species may eventually be the best models for the study of the viral etiology of human cancers. Two Type C viruses isolated from a sarcoma of a Wooley monkey and from a lymphoma of a Gibbon ape can be propagated in cell culture in amounts sufficient to provide important immunodiagnostic reagents. They have a common group antigen and a polymerase with properties characteristic of tumor viruses, but distinguishable from Type C viruses of lower animals. Similarly, a spontaneous mammary carcinoma which arose in a Rhesus monkey was found to contain appreciable amounts of a virus resembling the Type B particle causing murine mammary tumors. The virus not only replicates in monkey embryo cells but is infectious for cultured chimpanzee and human cells. The particles contain a polymerase similar to that found in RNA tumor viruses. Tests for oncogenicity in the Rhesus monkey are not yet well enough along to expect results. The finding of Type C and Type B viruses in tumors from old and new world primates greatly increases the likelihood that related viruses will also be found in human tumors.

Considerable effort was expended in the search for RNA viruses which might be oncogenic for man. Type C viruses resembling those of murine leukemia were discovered in the lymph nodes and blood plasma of leukemic patients. Since identification of free particulates as viruses is somewhat subjective, budding mature forms in tumor cells were sought with limited success. Although free virus or virus-like particles appeared occasionally in cell cultures, regular release of sufficient numbers of particles for study was not achieved. A culture originating from a liposarcoma apparently did release Type C particles, but virus production was not

sustained. For the past several years, sufficient numbers of observations reporting the finding of particles similar to the Type C and Type B in human malignancies have been made to conclude that viruses of these types may also infect man. The occasional presence of mature virus particles in human tumors, however, may be the exception rather than the rule.

With the development of new immunological and biochemical methods to detect Type C and Type B viruses or virus expression (polymerase activity assay, radioimmunoprecipitation inhibition assay, etc.), the finding that thymidine analogs (BrdU) are efficient virus inducers, and recent advances in molecular biology, the possibility of finding virus or virus expression in human tumors has increased by several orders of magnitude. Within the last year alone, four candidate RNA tumor viruses have been isolated and are being propagated in cell culture: ESP-1, RD-114, "Stewart virus", and a virus from the milk specimen of a woman with breast cancer. At the present time only two of these viruses are well enough characterized to describe further. ESP-1 was first isolated from cultures of cells in the pleural effusion from an American-type Burkitt's lymphoma patient. Investigations on the true origin of this virus are still under way and should be resolved soon. RD-114 virus was isolated from culture of a brain tumor of a fetal cat inoculated with human rhabdomyosarcoma cells. Intensive studies have already shown that the virus lacks the gs-1 antigen and shares the gs-3 antigen of known mammalian Type C viruses; the polymerase is related to the enzymes of the recently isolated primate Type C viruses. More data are obviously needed to confirm its human origin.

The finding that RNA tumor viruses of chickens or mice contain an enzyme with the property of assembling deoxyribonucleotides into specific DNA in the presence of an instructing RNA opened a new approach to the study of virus-induced cancer. Biochemists had known only enzymes capable of copying the genetic information contained in the nucleotide sequences of DNA into DNA or RNA copies. Soon it was demonstrated that reverse transcriptase was present in all the RNA-containing tumor viruses derived from many different animal species. The availability of substantial quantities of AMV permitted development of procedures for purification of this enzyme. It has been purified several hundredfold and shown to consist of a complex of two proteins having molecular weights of 110,000 and 69,000. The enzyme associated with other cancer viruses has been purified and shown to have generally similar properties. Many features of how the enzyme operates at the molecular level have now been elucidated.

Three findings from these studies appear to merit special mention in view of their practical consequences. First, a simple procedure for simultaneously detecting both the unique high molecular weight RNA and the enzyme reverse transcriptase of cancer viruses has been developed. This simple and rapid biochemical test provides a method for detecting cancer viruses and powerfully augments the much less sensitive and slower electron microscope examinations previously employed. The test has already shown utility in investigations of the role of viruses in human cancers.

Second, other studies indicate that the reverse transcriptase enzymes isolated from different cancer viruses have distinct immunological characteristics which are useful for typing the virus as to function (e.g. breast cancer, leukemia virus) and species of origin. This finding, combined with earlier data on other type-specific and interspecies group-specific antigens of cancer viruses, provides a range of serological characteristics for classification of suspected human cancer-virus candidates. Third, it has been shown that the reverse transcriptase enzyme from AMV can be used to prepare DNA copies of the RNA of other cancer viruses: DNA complementary to the RNA of mouse leukemia virus and mouse mammary tumor virus has been prepared. The last finding derives its importance from the availability of specific hybridization procedures.

Within the past year applications of these three new procedures arising from studies on animal viruses have developed significant new data relating to human cancer problem. About a year ago evidence from electron microscopic studies was presented that human milk specimens, particularly those obtained from women whose family histories would indicate increased risk to breast cancer, contained particles similar to the known mouse mammary tumor virus. It was then shown that the presence of such particles coincided with the presence of detectable reverse transcriptase activity in human milk. The procedure was also used to demonstrate the presence of appropriate RNA and reverse transcriptase in purified viral particles isolated from human milk specimens, and to monitor and establish conditions for the successful infection and propagation of the suspected human breast cancer virus in human cell cultures. Preliminary results from these studies seem promising.

In a study of mouse mammary tumors induced by MTV, DNA complementary to MTV RNA, made from MTV-RNA with the aid of AMV reverse transcriptase, specifically hybridized to the RNA obtained from mouse breast tumors. The RNA used in these experiments was isolated from the microsomal fraction of the tumor cells and thus presumably contained messenger RNA in the process of being translated into protein. The DNA failed to hybridize to the RNA isolated from the microsomal fraction of normal mouse breast tissue. The DNA also failed to hybridize to the RNA isolated from mouse leukemia virus but did, as expected, hybridize to the RNA obtained from MTV.

These studies were extended to examine human breast tumors. Nineteen of 29 specimens of human breast cancers yielded microsomal fractions which hybridized with DNA complementary to mouse MTV RNA. These microsomal RNA fractions failed to hybridize to DNA complementary to mouse leukemia virus RNA. Normal breast tissue specimens or breast tissue from various benign lesions gave microsomal RNA fractions which did not hybridize with DNA complementary to MTV.

This approach was then extended to the study of human leukemias and sarcomas. It was observed that the white blood cells of 24 out of 27 leukemic patients examined yielded polysomal RNA which hybridized to DNA

complementary to the mouse leukemia virus, but not to the DNA prepared from unrelated viruses causing mammary tumor in mice or myeloblastosis in chickens. No control human white blood cells or other adult or fetal tissues showed significant levels of the leukemia-specific RNA. Eighteen of 25 specimens of microsomal RNA from human sarcomas hybridized to the DNA complementary to mouse leukemia virus RNA, but not to the DNA complementary to mouse MTV.

The above findings suggest that human breast tumors contain functional genes which are related to the genes contained in the virus known to induce mammary tumors in mice. Human leukemias and sarcomas also appear to contain different functional genes related to the viruses known to induce leukemias and sarcomas in mice and other animals. In each case, the viral-related genes in the human tumors are functional in that some proteins are presumably being synthesized. At this time, it is not known if all the proteins required for production of complete viruses are being synthesized or whether synthesis is restricted to include certain proteins which are involved in the cancer transformation of the cell. It can, however, be anticipated that efforts will be expanded in the coming year to determine the possible implication of both the virus particles obtained from human milk and the RD-114 virus in human cancers.

DNA VIRUSES

Characteristics. There is also evidence linking the etiology of several human tumors to DNA viruses, especially those of the herpesvirus group. As with RNA tumor viruses, diseases of animals have provided useful model systems. Herpesviruses recovered from wild rabbits, chickens, and higher primates induce lymphoproliferative neoplasias in their respective hosts and the association of a virus from frogs with kidney carcinoma is well known.

In general, herpesviruses can show either productive or non-productive growth cycles. Cell death invariably accompanies the production of virus particles and the course of this production is similar for all members of this group. In common with other oncogenic DNA viruses of animals, certain herpesviruses stimulate cellular DNA synthesis and transform cells with finite growth capability into cultures with infinite growth potential. But, herpesviruses like the RNA tumor viruses also establish persistent covert infections. There is no definite evidence that they are vertically transmitted *in vivo* in a manner analagous to RNA tumor viruses, but transmission of the genome present in non-productive, cultured cells to progeny can occur. Activation of virus production also occurs in "virus-free" cells after exposure to mutagens (BrdU) and irradiation, supporting the idea that this group of viruses, although normally cytocidal, has oncogenic potential.

The question has been raised as to whether such herpesviruses produce their effects by expression of genes different from those involving cancers induced by RNA containing viruses, or whether such herpesviruses may indeed not be intrinsically cancerous but produce tumors through an

aberrant response of the infected host. Such tumors are now being examined for the expression of viral-related genes.

Relationship to human tumors. Over the past several years, viruses of the herpes type have been associated with certain human cancers: Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin's disease, infectious mononucleosis, and cervical carcinoma.

A herpesvirus, the Epstein-Barr virus (EBV), may be a contributing factor in the genesis of Burkitt's lymphoma. The mode of infection and site of production of EBV in this disease is unknown. Immunoglobulin production shows that Burkitt's lymphoma cells are monoclonal in origin, indicating that the tumor may not be of multifocal origin. A virus cause of this tumor is suspected because it occurs in high incidence in regions of Africa favorable for insect-vectored disease. Moreover, cases in certain areas cluster in time and space. EBV has been regularly isolated from tumor tissue, suggesting this virus may be the cause of the disease. Most patients with tumor have high titers of antibody to the viral antigens. However, a large proportion of normal people of all ages have antibodies to the virus, though usually in low titer. Further studies have shown that primary EBV infection of young adults caused some forms of infectious mononucleosis. Therefore, EBV may simply be a passenger within lymphoma tissue, isolated with regularity because it has a predilection of the lymphocyte and is widely distributed in humans. Existing information does not permit resolution of the question, but several aspects of the association of EBV infection with this tumor support a more than passive role. The high titer of antibodies in patients with Burkitt's tumor is not characteristic of other lymphoproliferative conditions. Even though 95 percent of normal African children may have antibodies, one would expect that a few tumor cases would be sero-negative were virus infection not an integral feature of the disease.

The lymphoma patient possesses a greater spectrum of antibodies to the several antigenic components in virus-infected cells than does the normal individual. The presence of certain antibodies appears to be related to the condition of the patient with respect to tumor. Antibodies to the membrane antigens in EBV infected cells as well as precipitating antibodies fell to negligible titer when a treated patient was followed during tumor regression and rose when tumor recurred months later. The recently isolated EBV-induced early antigens (EA) were found to be more disease associated than the viral capsid antigen. Two antigenic components were differentiated in the EA complex by indirect immunofluorescence tests: the D or diffuse staining complex and the R or restricted complex. For prognostic purposes, the presence or absence of antibodies to the early antigens appears to have important implications. The serums of a majority of patients with Burkitt's lymphoma contain antibody to the R antigen; the absence of antibodies is a favorable sign.

The response of lymphocytes to infection also suggests that EBV may have some attributes of the oncogenic RNA viruses. Lymphocytes from EBV negative donors fail to grow in continuous culture unless they are exposed to

infectious EBV. This apparent cellular transformation is attended by chromosomal aberrations, a feature associated with herpetic infections. Not all long-term lymphoblastoid cell cultures release EBV and may not contain antigens demonstrable by immunofluorescence tests. Nevertheless, the presence of EBV antigens has been detected by serologic tests and by homology of cellular DNA and mRNA with viral DNA. Such evidence of covert infection has been extended to biopsies of tumor tissues. Primary tumor rarely shows any cells positive for EBV by immunofluorescence, but all tumors examined have contained DNA homologous to viral DNA. Clones of EBV positive cells release virus as did the parent culture. Evidently, the virus genome persists in cells, frequently with minimal antigenic expression, and is transmitted to progeny during cell division. Thus, biologically this virus resembles the RNA tumor viruses.

While there is good reason to believe that EBV is involved in the oncogenic process, its role could be that of an accessory factor. High antibody titers to virus antigens are consistently found in patients with diseases other than Burkitt's tumor. These include nasopharyngeal carcinoma, the sarcomatous form of Hodgkin's disease, and some cases of sarcoidosis. Although examples of a causal relationship between one virus and different manifestations of malignant disease are known in avian and mammalian systems, the situation with regard to EBV is one of apparent rather than demonstrable direct association.

High EBV antibody titers, at least in some disease states, may be secondary to the disease process. In fact, not every individual bearing Burkitt's tumor has a high anti-EBV serum titer. Tumors of the Burkitt type do occur in temperate regions. Therefore, some combination of environmental factors unique to Africa does not appear to be an essential feature in the etiology of this disease.

If we assume that infection by EBV primes the cell for neoplastic change, it is possible to conceive that one or more combinations of environmental and host factors could interact to promote oncogenesis. Thus, the association of EBV infection with more than one disease becomes more plausible. Should EBV prove to be a necessary co-factor in any or all diseases with which it appears to be associated, control of infection by this virus would be of paramount importance. The prospective seroepidemiological study on a sizeable population of children in an area where tumor occurs with relative frequency should do much to clarify issues. The availability of an animal host susceptible to infection by EBV would also aid in the study of this virus.

Genital strains of Herpesvirus hominus Type 2 might be a contributing factor in carcinoma of the uterine cervix. Seroepidemiological studies conducted in several cities in the United States showed that significantly more women with invasive carcinoma have antibodies to this virus than do women without this tumor. The incidence of this neoplasm is related to sexual experience. The cancer is rare in nuns and most prevalent in promiscuous women, where the exposure to infection by the virus is expected. When these studies were extended to selected groups of women

in other countries, the results were not as conclusive. The question arises whether the methods applied are not sufficiently discriminating to permit detection of antibodies to certain genital strains in the presence of antibodies to oral strains of the virus, or whether the tumor-virus relationship is simply fortuitous. According to some investigators, there remains a significant difference between the rate of herpesvirus Type 2 antibodies in cervical patients compared to controls and further epidemiological studies will continue in this area.

RNA Viruses

Animal

Simultaneous activation of Type C RNA tumor virus expressions (most often gs antigen, occasionally infectious virus) occurs in chemically-induced as well as spontaneous tumors. With the development of the extremely sensitive radioimmune precipitation inhibition (RIPI) test, most of the tumors which were gs negative by complement-fixation were positive for gs antigen. Coincident switch on of the major species-specific protein peptides of the RNA virus in tumor cells produced by a variety of chemical carcinogens provides evidence that the carcinogens may act in cells to derepress the RNA viral genome known to be present in mouse cells.

Previous studies showed that rat and hamster cells infected with RNA tumor viruses provided extremely rapid, sensitive, and reproducible transformation assay systems for carcinogenic chemicals. After testing large numbers of non-carcinogenic analogs together with their related carcinogens in a standardized rat Rauscher leukemia virus-infected cell system, the positive and negative results agreed remarkably well with the known in vivo carcinogenic activity of these compounds.

A number of investigators have now shown the presence of a reverse transcriptase in all known oncogenic viruses. In order to apply this finding to human cancers, methods are being developed to distinguish viral from cellular polymerases. One approach being pursued is the use of specific antisera to inhibit polymerase activities of various classes of oncogenic viruses. This technique has provided an extremely sensitive immunoassay not only for the detection of tumor virus expression in cells but also for distinguishing Type B (mammary tumor) from Type C (leukemia/sarcoma) viruses.

A rapid, sensitive assay has been developed to detect RNA-DNA and DNA-DNA hybrids by the use of nucleases (Aspergillus sp., mung bean) that will degrade only single-stranded nucleic acids, leaving hybrid structures intact. This assay could eventually replace the more costly and time-consuming hydroxyapatite chromatography or $CsSO_4$ equilibrium centrifugation methods.

A genetically stable change in host range and the development of new viral surface antigens occurs in an animal RNA tumor virus after prolonged passage in human cells. The most exciting interpretation of these findings is that recombination has occurred with a Type C human virus, which itself may exist in an integrated form within the host cell genome. The new viral surface antigens would then reflect the genetic information of the latent virus and could be useful markers to search for evidence of a viral etiology of human cancer. These observations also emphasize that caution must be exercised in evaluating new viruses isolated from human cancers since they may in fact be animal viruses with human "outer coats."

Immunogenetic studies on recombinant inbred lines of mice have identified the genes responsible for host response to both exogenous and endogenous virus expression, replication, and tumorigenesis; and the host range of tumor viruses. The gene responsible for gs-antigen expressio expression of a murine leukemia virus is transmitted as a simple Mendelian dominant. Another gene has been identified as a determinant of virus replication and segregates independently from the gs-determinant gene.

Molecular hybridization experiments employing the DNA products of avian and murine tumor viruses and DNA of normal and tumor cells show that DNA copies of the virus produced by the reverse transcriptase sequences are present in normal as well as tumor cells, regardless of whether they have infectious virus. Normal cells, therefore, contain part or all of the sequences necessary to make RNA viruses.

A new test which is 1,000 times more sensitive than complement fixation and 40,000 times more sensitive than immunodiffusion for measuring viral protein is so specific that any sera with reactivity against the gs-antigen of animal leukemia viruses can be employed regardless of the other reactivities which are present. The test is currently being applied to a number of studies on animal tumor viruses.

Structural studies on the 70S RNA of avian oncogenic viruses indicate that the 70S molecule consists of 35S subunits. These subunits are linked by hydrogen bonds to form the 70S complex. Two size classes of subunits can be resolved by electrophoresis in polyacrylamide gels. The larger class has been found exclusively in sarcoma viruses whereas the smaller class occurs in sarcoma and leukosis viruses. This evidence suggests that RNA subunits contain genetic information which is required for transformation of fibroblasts; the subunits of tumor virus RNA can therefore be considered as functionally specialized.

Studies on the RNA-dependent DNA polymerase suggest that this enzyme functions as a repair enzyme to produce a complementary copy of a single strand of RNA by building from the terminal end of a primer strand linked to the template strand. This finding suggests that this enzyme, present in virions, is not directly involved in the replication of the RNA of tumor viruses.

Antigens of the Mason-Pfizer monkey virus (M-PMV), previously isolated from a breast cancer of a Rhesus monkey and shown to contain both 70S RNA and reverse transcriptase, have been found to be present in two of five normal Rhesus monkey embryos. This finding suggests that M-PMV may be indigenous and vertically transmitted in this species.

A method was developed permitting the detection of two markers of RNA tumor viruses, the 60-70S RNA and reverse transcriptase activity, within crude specimens containing relatively small numbers of particles. It is now possible to characterize particles detected in specimens from human cancer patients in terms of their content of high molecular weight RNA and of RNA dependent-DNA polymerase.

Highly purified (electrofocused) species-specific gs antigens and antisera have been prepared for the RNA tumor viruses of the following species: mouse, rat, cat, hamster, human? (RD114), viper and chicken; each one is species-specific in gel diffusion, radioimmune precipitation inhibition, and complement-fixation.

A Type C virus, continuously released by the R-35 cell line established from a spontaneous mammary carcinoma of the rat, infects and transforms cells. When implanted into rats, these cells produced tumors of possible mammary gland origin.

Two viruses have recently been isolated from non-human primate tumors (woolly monkey, gibbon ape). They appear to be distinct from other known animal tumor viruses and may be the best models for study of the viral etiology of human cancers. Isolation and characterization of the viral antigens, including group-specific antigens and polymerase, will provide important immunodiagnostic tools for the characterization of new human virus isolates.

Viral markers (gs-antigens and RNA directed DNA polymerase) can be activated by alteration of the physiological endocrine balance. This finding lends support to the concept that genetic formation for tumor virus synthesis and possibly tumor formation is transmitted vertically and can be activated by definable physiological mechanism.

Viruses activated and recovered from normal and tumorous mouse tissue, and from mouse cells (normal and transformed) grown in tissue culture were tested for oncogenicity. Wild type RNA viruses and their subinfectious virus expressions were shown to have oncogenic potential.

Sera from over 80 cats with spontaneous or virus-induced lymphoma or sarcoma were all negative for antibody to species-specific gs antigen in complement fixation and radioimmunoassay precipitation inhibition tests, thus confirming previous studies suggesting that cats are immunologically tolerant to this antigen.

Candidate Human

A Type C virus (RD114) originated in RD human rhabdomyosarcoma cells after passage of this cell line through a fetal cat. Immunological studies showed that the virus had none of the species-specific (gs-1) properties of the cat, mouse, rat, hamster, chicken, or viper Type C viruses. The gs-3 antigen of RD114 showed cross-reactivity with the mammalian RNA tumor virus interspecies specific-antigen. The polymerase of RD114 was shown to be more closely related to the Type C viruses isolated from primate tumors than to the tumor viruses of lower mammals. Hybridization studies with cat virus DNA showed no significant homology with RD114 virus; RD114 virus showed an especially high degree of homology for RNA of Hodgkins cells. These preliminary studies will require confirmation before the human nature of the RD114 virus can be accepted.

The ESP-1 cell line obtained from an American child with Burkitt's lymphoma continued to release particles resembling a Type C virus. Reports vary on the antigenic relationship between this virus and the gs-1 antigen of mouse leukemia virus. A gs-2 antigen detected in the ESP-1 virus was considered identical to gs-2 antigen of a murine tumor virus. The virus possesses the gs-3 interspecies antigen common to the known mammalian leukemia viruses and a polymerase closely related to the MLV(Rauscher).

Cell cultures of a human rhabdomyosarcoma induced with IrdU produced Type A particles. Except for their intracisternal location, many of them had dense nucleoids and resembled mature Type C virus particles. This reaction was enhanced by a factor of 10 when treatment with IrdU was followed by treatment with DMSO. A second culture established from a metastatic adenocarcinoma of a bronchial node and treated with IrdU and DMSO not only showed intracisternal Type A, but also typical Type C virus particles that budded from the plasma membrane into extracellular spaces.

Polysomal RNA extracted from the leukocytes of leukemia patients and from human sarcoma cells was shown to possess homology with nucleotide sequences in the RLV genome but not with the RNA of the AMV or MTV. No similar significant homology could be demonstrated with polysomal RNA from human mammary carcinoma cells or other control tissues. Genetic information similar to that contained within the RLV genome, but not within AMV or MTV, has been found in human neoplasias resembling those associated with RLV infection in animals. In this respect, a common genetic factor appears to be present in neoplasms arising in tissues of mesenchymal derivation.

Particles resembling Type C animal tumor viruses were detected in a tumor and cell cultures established from a human fibrosarcoma. Treatment of the culture with IrdU increased the number of virus particles observed in the growth medium. This finding provides additional suggestive evidence that viruses of the Type C may be of significance in the etiology of certain human neoplasms.

A continuous cell line, HBT-3, was established in culture from a human breast adenocarcinoma. The cells are epithelioid in appearance, grow rapidly, and form multilayered colonies. Since there are very few

continuous human breast tumor lines in existence, this cell line should be useful in studies on the relationship of RNA tumor viruses to human breast cancer.

Recent data indicate that there is no correlation between the finding of virus particles (Type B) in milk specimens and increased risk to develop breast cancer.

DNA Viruses

Animal

In a study of the possible etiological relationship of Herpesvirus Type 2 to cervical carcinoma, tumor biopsies were examined for evidence of viral infection. Although no evidence of virus particles or antigens was found in the biopsies or in tumor tissue cultures, one line, cultured for several months, was found to release virus when the pH of the medium was increased substantially. This virus now has been shown to be perpetuated within the cells of this tumor without overt expression, demonstrating that non-cytolytic infections do occur naturally.

Herpesvirus saimiri (HVS), a virus inducing a lymphoproliferative disease in squirrel monkeys, can now be studied to determine its natural mode of transmission and pathogenesis in different primate species. The virus is widely distributed in squirrel monkeys and can be isolated readily from blood samples. These observations suggest that the agent is highly infectious and transmitted horizontally within this susceptible monkey population. Recently over 30 owl monkeys inoculated with HVS have consistently developed malignant lymphoma and lymphocytic leukemia. Usually more than one injection of virus was needed to induce disease. No Old World monkeys have developed tumors over a period of two years. This virus presents a primate model to study oncogenicity by herpes-virus infection in a species closely related to man.

Herpesvirus Type 2 isolates treated by UV irradiation induce transformation of hamster cells; Herpesvirus Type 1 strains subjected to similar treatment do not show transforming properties. Attempts to demonstrate the presence of other viruses or virus expression (RNA tumor viruses) in these cells which might have contributed to the transformation have been unsuccessful. Thus, a genital strain of herpesvirus which has been suspected to contribute to the development of human cervical carcinoma has been shown to induce neoplastic changes in animal cells.

"Oncogenic" DNA virus transformed mouse cells were transplanted into newborn Swiss mice. The resulting tumors were strongly positive for gs antigen but negative for infectious virus. Since the DNA virus does not cause tumors in the natural host, these findings favor the hypothesis that neoplastic changes produced in heterologous cells and animals by these viruses are due to derepression (or activation) of the RNA virus genome.

EBV was activated in cells of the "virus-free" Raji strain of Burkitt's lymphoma by treatment with BrdU. This procedure should make it possible to screen large numbers of lymphoblastic leukemia cell cultures for covert EBV virus. It may also help determine whether all virus-free Burkitt cell clones contain the genome.

Under germ-free conditions, chickens which appear to be free of leukosis viruses respond differently from conventional chickens which develop typical symptoms of Marek's disease when infected with Marek's disease virus. The fact that germ-free birds succumb to infection with lesions of the central nervous system suggests that Marek's disease may represent an interaction between the host, Marek's disease virus, and a leukosis virus.

Candidate Human

Recent preliminary findings stemming from the discovery of unusual clusters in the Albany, N. Y. area lend credence to the possibility that Hodgkin's disease may be infectious. There is no evidence, however, that this disease as it occurs in man is of a contagious nature. Virologic studies have concentrated on the possible role of EBV in Hodgkin's disease. Patients with this disease showed high antibody titers to EBV, similar to those found in patients with Burkitt's lymphoma. The results must be interpreted with caution since elevated antibody titers may be the result of the disease rather than a reflection of its etiology.

Two new early antigens of EBV have recently been described. The D antigen (methanol resistant) appears first in the nucleus of infected cells and later in the cytoplasm; the R antigen (methanol sensitive) appears only in the cytoplasm. Serums of patients with infectious mononucleosis and nasopharyngeal carcinoma have antibodies that react with the D antigen, whereas serums of Burkitt's lymphoma patients have antibodies to the R antigen. For prognostic purposes, the presence or absence of antibodies to the early antigen appears to have important implications. At least in the case of Burkitt's lymphoma, absence of antibodies to the R antigen is a favorable sign.

Treatment and Control

A new category of "unblocking or deblocking" antibodies that abrogate blocking antibodies was found in the sera of patients whose cancers were surgically removed or in remission.

An increase in the immunogenicity of weak tumor (SV40, MC transformed) cell surface antigens was obtained by incorporating influenza virus antigens into the cell surface membranes. Following formalin treatment to inactivate the influenza virus, vaccination of animals with homogenates of these cells gave significant protection to animals against challenge

with viable cells of that tumor.

Streptonigrin in nanogram doses is highly effective in inhibiting replication of murine leukemia and sarcoma viruses in vitro. A marked decrease in titer of MLV(Rauscher) recoverable from infected mice as well as an increase in survival time of these mice was observed in those groups treated with this antibiotic.

Sera and lymphocytes from several thousand cancer patients were assayed in cytotoxicity tests for reactivity to more than 60 different cultured tumor cells having 25 separate HL-A antigens. The studies suggest that a genetically susceptible population of individuals exists with a higher risk of developing Hodgkins disease, cervical cancer, and lung cancer.

Although Rifampicin and the related antibiotics are inactive, certain 3-substituted Rifamycin SV derivates are potent inhibitors of cancer-virus reverse transcriptase. Most of the active derivatives also inhibit other DNA and RNA polymerases normally present in the mammalian cell and thus are toxic, but a few do show a degree of selectivity against particular enzymes. A few compounds appear to show selective toxicity for leukemic lymphocytes as compared to lymphocytes from normal human donors. The possibility of molecular control of cancer through tailoring of compounds aimed at either killing cancer cells or reverting them to normal behavior through blockade of specific gene functions associated with cancer appears promising.

AKR mice have been considered immunologically tolerant to GMuLV, since they are G antigen positive during their entire life span. Recently, kidney-eluates of untreated AKR mice were found to contain at least two different antibodies to intraviral G antigen(s) and GCSA, although no free antibodies are detected in the body fluids. These findings indicate that there is no absolute immunological tolerance in the Gross leukemia system.

Immunization of AKR mice with neuraminadase treated thymocytes was effective in the prevention and control of leukemia in this species. This finding indicates that tolerance in AKR mice may not be complete and that treatment regimens of chemotherapy and immunotherapy may be effective.

D. Projections:

Last year, the Viral Oncology Area set forth a long range research plan for the identification and control of virus-induced cancers of man. Studies within the following broad categories have already been implemented and will be expanded in the coming year:

1. Virus (or virus-expression) - tumor relationships

   a. Model Studies. Studies on animal, RNA and DNA, tumor viruses, known to cause malignancies in several mammalian species, will be continued. The results of these studies have already provided important information about tumor viruses that is applicable to the isolation and identification of human agents. Special emphasis will be given to determine the characteristics of several newly-isolated primate viruses. This work will remain an integral part of the Program.

   b. Human Studies. Efforts to identify viruses or detect virus expression in human tumors have been underway for some time. Because of this year's successes in isolating candidate human viruses, the Program will continue to increase its activities in the search for viruses which induce malignancies of man.

      (1) To identify and isolate candidate viruses or subviral products in leukemias, lymphomas, sarcomas, and carcinomas (breast).

      (2) To identify and isolate candidate viruses or subviral products in lung, colon, and other carcinomas.

      (3) To develop methods for the detection of high cancer risk groups, i.e. individual susceptibility or predisposition to transformation by human viruses.

      (4) To extend present and develop new methods for the successful propagation of significant amounts of human candidate viruses.

      (5) To extend existing and develop new methods to induce tumor virus or virus expression in "normal" cells.

      (6) To develop suitable reagents and to modify existing immunological and biochemical methods for mass diagnostic screening for candidate viruses.

      (7) To characterize, biologically and biochemically, presumptive viral agents.

      (8) To increase emphasis on understanding the relationship of environmental agents (e.g. chemical carcinogens) as co-factors in viral carcinogenesis. This represents a

major expansion of programs requiring combined skills of the Viral Oncology and Chemical Carcinogenesis Areas.

2. Molecular Studies

Major advances in the understanding of the molecular pathways of tumor virus replication have been made within the past year. They have already provided the basis for new, extremely sensitive methods for the detection of oncogenic viruses or virus expression. It is now possible to characterize agents detected in specimens of human cancer patients in terms of their content of high molecular weight RNA and of RNA-dependent DNA polymerase. Specific hybridization procedures already provide a method for further investigation of host-cell-virus relationships which have been extended into the study of human cancers. Preliminary results offer strong supportive evidence that certain human tumor cells contain genetic information related to that found in known oncogenic viruses.

a. Basic Studies

The Program will continue to broaden its activities for detecting, identifying, and characterizing the spectrum of enzymes and their products required by tumor viruses for replication and/or transformation.

b. Applied Studies

As knowledge of the fundamental molecular events in virus-cell interactions mounts, the Program will continue to apply this information to the study of human cancer as follows:

(1) To identify and characterize similar enzymes or enzymatic activities within normal and malignant human cells.

(2) To develop highly sensitive methods for the detection of virus or virus activity in human cells.

(3) To develop a rational basis for therapy or prevention by exploring various approaches to blocking of viral replication and/or tumorigenesis at the cellular and subcellular levels. The therapy could be directed at any or all of the stages of cell transformation beginning with cell infection by a tumor virus.

Ultimately these approaches will require an intensified program to develop drugs, anti-enzymes, gene repressors, or inhibitors effective at the molecular level. This represents a major expansion of effort requiring the combined skills of the Viral Oncology and the Chemotherapy Areas.

3. Immunological Studies

Immunologic research has provided extremely sensitive techniques for detection and characterization of tumor viruses, viral antigens, and changes in surface membranes of tumor cells. These studies have also contributed to an understanding of the role of immunological mechanisms in host-tumor and host-virus interactions which provide a rational approach to the prevention and treatment of cancer.

a. Basic Studies. Investigations of selected model systems, representing tumors induced by Type C, Type B, and Herpestype viruses, will be extended to further identify, characterize, and determine the viruses, viral antigens, and membrane antigens of tumor cells. The studies include development and application of improved techniques to detect cellular alterations induced by tumor viruses alone or as the result of interaction with other environmental agents. Increased emphasis will be placed on research on spontaneous or naturally occurring tumors in model systems relevant to human cancer, as a basis for a rational approach to prevention and treatment.

(1) To study cellular and humoral immune mechanisms and to determine their relative significance in host recognition of and response to tumors and/or tumor viruses.

(2) To develop methods to enhance host response to tumors or tumor virus antigens.

b. Applied Studies. As basic research provides the framework for identification and characterization of viruses, viral antigens, and cell membrane alterations in human cancers, immunological methods will be applied:

(1) To relate candidate human viruses to known oncogenic agents.

(2) To identify and characterize intra- and inter-species viral antigens which are present in known mammalian tumors, as probes for detecting human tumor viruses or viral antigens

(3) To determine the presence of cross-reacting antigens in various human tumors.

(4) To launch large-scale seroepidemiological surveys which will define high risk populations.

Clinical studies will be directed toward understanding and manipulation of immune mechanisms in human cancer as a basis for:

(1) Development of vaccines from identified and

fully characterized human tumor viruses.

(2) Determination of the role of host immune responses in tumor recognition and rejection.

(3) Application of (1) and (2) in the prevention and control of human cancer.

Application of the results of these studies to human cancers will require the coordinated skills of the Viral Oncology and General Laboratories and Clinics Areas.

4. Test Systems

In vitro and *in vivo* (animal) test systems will be carefully selected to evaluate the work outlined in the previous research areas:

a. To determine the oncogenic potential of candidate human viruses;

b. To develop bioassay systems for testing viral, and viral/chemical carcinogens;

c. To begin vaccine (conventional or other) testing and immunization programs;

d. To begin therapy testing programs;

e. To explore special animal tumor systems, especially in primate species particularly relevant to human cancer;

f. To develop and maintain well-characterized cell culture lines and animal stocks (small mammalian and primate species).

Many of these systems will be developed at the National Cancer Institute's Fort Detrick facility.

5. Resources

Since research efforts undergo continual change in emphasis and scope as new leads emerge, a variety of resources will have to be developed, maintained, and coordinated.

a. Human Resources - collection and storage of serum and tissue specimens, integration of data on clinical records, storage and distribution; computerization, coordination of specimen collection, storage, and distribution;

b. Animal Models - maintenance of various mammalian animal colonies for basic research and special studies;

c. Reagent Production - large scale production of animal tumor viruses for basic research; production of standardized lots of purified viruses; and production of high quality diagnostic reagents;

d. Candidate Human Virus Production - intensive developmental research effort to isolate and produce human viruses;

e. Biohazards Control and Containment - controlled environment facilities are required for research on known oncogenic viruses and candidate human tumor viruses as well as for maintaining animal colonies which are protected from extraneous infections.

To fulfill the continually increasing need, resources have been placed under the control of a new Office of Program Resources and Logistics. Within the coming year, a prime contractor may be required to assist in the expansion of this important task.

FUNDING LEVELS OF CONTRACTS WITHIN SEGMENTS
(By Type of Institution)
Viral Oncology Area, Etiology, NCI

Segment	Profit		Educational		Non-Profit		Other*		Total	
	No.	Amount**	No.	Amount	No.	Amount	No.	Amount	No.	Amount
Program Management	5	2,944	15	1,470	3	85	2	1,680	25	$6,179
Developmental Research	3	1,875	16	4,718	7	955	0	0	26	$7,548
Solid Tumor Virus	3	5,173	12	5,442	5	950	2	235	22	$11,800
Immunology	2	257	5	312	5	903	1	150	13	$1,622
Resources and Logisitics	14	7,113	13	2,035	9	435	1	37	37	$9,620
Breast Cancer Virus Studies	2	563	5	528	4	638	0	0	11	$1,729
Biohazards and Environ. Cont.	1	200	2	389	1	0	1	67	5	$ 656
TOTAL	30	18,125	68	14,894	34	3,966	7	2,169	139	$39,154

* Includes interagency agreements.
**Dollars in Thousands.

SPECIAL VIRUS CANCER PROGRAM

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July 1, 1971 to June 30, 1972

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SUMMARY SHEET

OFFICE OF BIOHAZARD AND ENVIRONMENTAL CONTROL

The Office of Biohazard and Environmental Control conducts research pertaining to the physiological and environmental factors that alter the hosts susceptibility and response to oncogenic and non-oncogenic viral infections. It also evaluates and develops techniques and equipment to minimize cross infection and provide adequate environmental controls.

The Biohazard Section conducts research to elucidate the mechanisms involved in host immunocompetence and the consequence of this on oncogenesis. Furthermore, understanding is being sought of the biochemical factors that lead to the induction of malignancy and how best to detect and modify these inductive factors. Physiological imbalances, induced by controlled stress are being examined in in vivo and in vitro systems in order to assess the host response.

We have for the first time demonstrated that the virogenic markers, group specific antigens (g.s.) and RNA directed DNA polymerase, can be activated by alteration of the physiological endocrine balance. This major finding lends strong in vivo support to the concept that genetic information for tumor virus synthesis and possibly tumor formation is transmitted vertically and can be activated by definable physiological mechanism. The potential for such activation has also been noted by us to be present in certain pesticides and in relatively low levels of ionizing radiation. We have also, for the first time, demonstrated that the host can be senitized to specific viral stimuli and subsequently demonstrates such cellular immunity by blastogenic transformation of lymphocytes.

The Environmental Control Section utilizes environmental monitoring to identify sources of contamination. To assure the integrity of primary and secondary biological barrier systems, to evaluate engineering and operational parameters and their effect on experimental and personnel contamination control. Similarly, they collect and have evaluated information pertinent to biological safety.

Minimum Standards for Biological Safety and Environmental Control have been issued to all contractors within the SVCP, these are currently being implemented. We have similarly initiated a centralized sera epidemiological surveillance service for all contract operations as well as having initiated a safety training program for senior laboratory technicians and project directors. Our consultation service to contractors, in-house research facilities and grant associated programs continues. We are planning to initiate a Chemical Carcinogenesis Biohazards Control program. This latter effort should reach full operation during the next reporting period.

Serial No. NCI - 4805

1. Office of the Associate Scientific Director, Viral Oncology, Etiology
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Parameters of Immunosuppression in the Malignant State

Previous Serial Number: Same

Principal Investigator: Dr. Alfred Hellman

Other Investigators: Dr. Arnold K. Fowler
Dr. Phyllis D. Kind
Dr. Harry G. Steinman

Cooperating Units: None

Man Years:

Total	2.5
Professional:	0.6
Others:	1.9

Project Description

Objectives:

The evaluation of host immunocompetence in controlling oncogenesis. This is being investigated at the genetic-biochemical level and by cellular immunological means.

Methods Employed:

Cellular immunocompetence of the host during oncogenic and non-oncogenic viral infection is being investigated by means of the host response to specific viral blastogenic stimulants. The host response to alteration in physiological environment is being similarly evaluated at the cellular and humoral immunological level by means of radio-isotopic and complement fixation reactions.

Major Findings:

We have for the first time been able to demonstrate specific blastogenic effects due to host sensitization by viral infection. We have also for the

first time demonstrated the activation of two separate tumor virus markers (g.s. and reverse transcriptase) in vivo by means of hormonal manipulation of the host. Similarly we are demonstrating that there are both quantitative and qualitative alterations in the in vivo host in species of tRNA brought about by tumor virus infection.

Significance to Biomedical Research and the Program of the Institute:

The elucidation of the molecular events leading to malignancy in vivo as well as its implication in immunocompetence will provide a more rational means for the control and therapy. An understanding of inducing or co-inductive factors associated with malignancy will provide for more meaningful means of control and prevention.

Proposed Course:

The development of in vitro assay system for studying early inductive changes during viral produced malignancy and to determine the genetic and immunologic factors associated with such inductions and its modification.

Honors and Awards:

Chairman at 1972 ASM meeting of seminar entitled: Biohazards in Viral Oncology.

Publications:

Hellman, A. and Fowler, A. K.: Hormone-Activated Expression of the C-type RNA Tumor Virus Genome. Nature New Bio. 233: 142 (1971)

Hellman, A., Fowler, A. K., Steinman, H. G. and Buzzerd, P. M.: Studies of the Blastogenic Response of Murine Lymphocyte. III. Specific Viral Transformation. Proc. Soc. Exp. Bio. Med. (in press)

Fowler, A. K., Hellman, A., Steinman, H. G. and Quatrale, A. C.: Studies on the Blastogenic Response of Murine Lymphocytes. I. Quantitative Measurement of Stimulation by Phytohemagglutinin. Proc. Soc. Exp. Bio. Med. 138: 345 (1971)

Steinman, H. G., Fowler, A. K. and Hellman, A.: Studies on the Blastogenic Response of Murine Lymphocytes. II. The effects of Serum-Stimulated-Cell Interactions on Phytohemagglutinin-Induced Stimulation. Proc. Soc. Exp. Bio. Med. (in press)

Fowler, A. K., Steinman, H. G., Reed, C. C. and Hellman, A.: Studies of the Blastogenic Response of Murine Lymphocyte. IV. Endocrinological Effects. Proc. Soc. Exp. Bio. Med. (in press)

Dunkel, V. C., Frenkel, L. D., Fowler, A. K. and Hellman, A.: Serological Patterns to EBV-Associated Antigens in a Patient With Infectious Mononucleosis. New Eng. J. Med. (in press)

Fowler, A. K., Reed, C. D., Todaro, G. J. and Hellman, A.: Activation of C-Type RNA Virus Markers in Mouse Uterine Tissue. Nature (in press)

Nettesheim, P., Hanna, M. G. Jr., Doherty, D. G., Newell, R. F. and Hellman, A.: Effect of Calcium Chromate Dust, Influenza Virus, and 100 R Whole-Body X Radiation on Lung Tumor Incidence in Mice[1,2]. JNCI 47: 1129-1144, 1971

Nettesheim, P., Hanna, M. G. Jr., Doherty, D. G., Newell, R. F. and Hellman, A.: Effects of Chronic Exposure to Air Pollutants on the Respiratory Tracts of Mice: Histopathological Findings USAEC Division of Technical Information Extension (AEC Symposium Series 21), Oak Ridge, Tennessee, 437-448, 1971

Serial No. NCI - 4806

1. Office of the Associate Scientific Director, Viral Oncology, Etiology
2. Office of Biohazard and Environmental Control, Environmental Control Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Evaluation of Biological Safety and Environmental Control Equipment, Facilities and Procedures

Previous Serial Number: Same

Principal Investigator: Mr. W. Emmett Barkley

Other Investigators: Dr. Alfred Hellman

Cooperating Units: None

Man Years:

Total:	2.2
Professional:	1.2
Other:	1.0

Project Description

Objectives:

The primary objectives of the biological safety and environmental control evaluation project are: (1) to establish an environmental monitoring program for the identification and resolution of biocontamination problems and to provide continuous surveillance of the primary and secondary biological barrier systems which have been incorporated into the National Cancer Institute's virus containment facility; (2) to evaluate engineering and operational parameters of prototype virus-cell culture laminar flow safety cabinets and to determine the effects

of those parameters on experimental and personnel contamination control; (3) collect and evaluate information pertinent to the biological safety aspects of procedures, equipment and facilities; (4) to develop a current inventory of agents studied in research laboratories supported by the Special Virus Cancer Program; and (5) to evaluate methods for handling potentially hazardous materials and infected animals.

Methods Employed:

Standard engineering methods for analysis of air flow characteristics and pressure differentials are employed. Physical and biological tracers are employed for determining the capability of facilities, equipment and procedures to control biological hazards under a variety of mechanical and operational stress situations. Volumetric air samples and airborne particulates are collected by standard environmental monitoring and industrial hygiene techniques.

Site visits to various virus laboratories associated with the Special Virus Cancer Program, using appropriate evaluation forms, are employed to observe the performance of safety procedures, equipment and facilities. Environmental monitoring surveys are performed to determine the degree and source of intramural biocontamination and to identify all factors which may create unsafe conditions. Data from the site visits and environmental monitoring surveys are evaluated to determine the capability of the laboratory to perform potentially hazardous research.

Major Findings:

Minimum Standards for Biological Safety and Environmental Control have been promulgated. These standards were approved by the OASDVO and transmitted to all SVCP laboratories.

The Environmental Control Section has prepared renovation proposal requirements for SVCP contractors who are anticipating space alterations. This document is intended to serve as a planning guide to assist the SVCP contractor in identifying his laboratory needs and defining his facility requirements. The document requirements necessitate a planning effort involving research, management, and architectural-engineering advisors.

Eleven formal site visits were performed under the Biological Safety and Environmental Control Survey Program. Reccommendations for improving the quality of the laboratory environments were developed and transmitted to the respective principal investigators.

Significance to Biomedical Research and the Program of the Institute:

This project will improve the quality and safety of research laboratories associated with the National Cancer Institute's Special Virus Cancer Program. Results from these studies will serve as a basis for establishing standard operating procedures for virus laboratories and appropriate design criteria for containment equipment. Currently there is a paucity of biological safety information pertinent to virus research. This project will contribute to alleviating this deficiency by disseminating practical biological safety information.

Proposed Course:

Twenty-five biological safety and environmental control surveys will be performed during the coming year. The surveys will include greater participation of the program project officers. The project officers will be asked to assist in solving environmental control and safety problems identified by the survey program.

Consultation activities will be expanded to assist the SVCP contractors in implementing the Minimum Standards of Biological Safety and Environmental Control.

The initiation of an applied research program has been planned. The development of relevant safety equipment and procedures will be emphasized.

Honors and Awards:

None

Publications:

None

Serial No. NCI - 4807

1. Office of the Associate Scientific Director, Viral Oncology, Etiology
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Immunocompetence and Susceptibility of Animal Systems to Oncogenesis

Previous Serial Number: Same

Principal Investigator: Dr. Arnold K. Fowler

Other Investigators: Dr. Alfred Hellman
Dr. Harry Steinman

Cooperating Units: None

Man Years:

Total	2.0
Professional:	1.0
Others:	1.0

Project Description

Objectives:

The objectives of this project are to define and characterize biological mechanisms contributing to host immunological regulation and to ascertain their role in oncogenic expression. Physiological imbalances, produced by controlled stresses, are to be examined at the biochemical level as initiators of host immunological inadequacy. In vivo and in vitro model systems will be developed to assess and monitor host immunocompetency.

Methods Employed:

The in vivo and in vitro activation of virogene marker expression by a variety of physiological stimuli is being characterized using conventional methods: group specific antigens of the C-type RNA tumor viruses (gs-1, gs-3) by complement fixation and gel precipitation techniques; virus synthesis by electron microscopy and "XC" assay; RNA directed DNA polymerase by biochemical and serological methods. These criteria of oncogenic detection are being studied concomitantly with various parameters

of host immunocompetence. These include the in vivo delayed hypersensitivity and graft vs host in vivo reactions and the in vitro response of lymphocytes to specific and nonspecific antigenic stimulation.

Major Findings:

The in vivo activation of virogene marker expression, the group specific antigens of the C-type RNA tumor viruses and RNA directed DNA polymerase, in mouse uterine tissue by endocrines has been described. The observation that this activation can be induced by hormones in physiological levels demonstrates, for the first time, a definable physiological mechanism by which viral genetic information may be derepressed and possibly repressed in an animal system. Additionally numerous other in vivo stimuli including exposure to synthetic compounds possessing hormonal activity as well as certain pesticides and radiation have been shown to influence virogene marker expression.

Further refinement of the lymphocyte culturing system described on last years annual report for studying murine cellular mediated phenomenon has led to the development of a "micro" culturing system. The further development of a fully automated "flow-through-filter" culture harvest device (patent application under 35 USC 266 has been filed) augments this system by permitting a very rapid and controlled means to selectively assay different parameters of lymphocyte cellular mediate recognition. Using this system various endocrines (steroids) associated with normal physiological functions and with virogene derepression and repression activity have been shown to alter lymphocyte blastogenesis.

Significance to Biomedical Research and the Program of the Institute:

The essential role of host immunity, as dictated by genetic and environmental control mechanisms, in the etiology of some, if not all cancers, has become increasingly apparent during recent years. The concept that degradation of natural immunity processes under certain environmental and/or physiological stresses precede or are concomitant with infection by oncogenic viral agents is not new, although it remains poorly defined. The frequent implication of host physiology in the development and pathogenesis of cancer, as well as their involvement in host immunocompetence, points to the need of further research directed toward elucidating the biochemical mechanisms and the degree of its participation in these phenomena as a prerequisite to the development of means to control human cancers.

Proposed Course:

The development of an in vivo system permitting the "at will" control of virogene marker expression by the manipulation of the host's endocrine milieu now provides a model to comprehensively examine the "oncogene" theory of tumorogenesis. Efforts will be concentrated on physiological imbalances as they interact with host genetics and immunocompetence in the induction and propagation of the malignant state. Furthermore

attempts will be directed toward the characterization of the underlying biochemical mechanisms of these interrelationship.

Honors and Awards:

None

Publications:

Fowler, A. K., Hellman, A., Steinman, H. G. and Quatrale, A. C.: Studies on The Blastogenic Response of Murine Lymphocytes. I. Quantitative Measurement of Stimulation by Phytohemagglutinin. Proc. Soc. Exp. Bio. Med. 138: 345 1971

Fowler, A. K., Reed, C. D., Todaro, G. J. and Hellman, A.: Activation of C-Type RNA Virus Markers in Mouse Uterine Tissue. Nature (in press)

Fowler, A. K. and Reed, C. D. Estrogen Analysis of Neonatal Bovine Urine Using Gas-Liquid Chromatography[1]. J. of Anim. Sci. (in press)

Fowler, A. K., Steinman, H. G., Reed, C. D. and Hellman, A.: Studies of the Blastogenic Response of Murine Lymphocyte. IV. Endocrinological Effects. Proc. Soc. Exp. Bio. Med. (in press)

Hellman, A. and Fowler, A. K.: Hormone-Activated Expression of the C-type RNA Tumor Virus Genome. Nature New Bio. 233: 142, 1971

Steinman, H. G., Fowler, A. K. and Hellman, A.: Studies on the Blastogenic Response of Murine Lymphocytes. II. The Effects of Serum-Stimulated-Cell Interactions on Phytohemagglutinin-Induced Stimulation. Proc. Soc. Exp. Bio. Med. (in press)

Hellman, A., Fowler, A. K., Steinman, H. G. and Buzzerd, P. M.: Studies of the Blastogenic Response of Murine Lymphocyte. III. Specific Viral Transformation. Proc. Soc. Exp. Bio. Med. (in press)

Dunkel, V. C., Frenkel, L. D., Fowler, A. K. and Hellman, A.: Serological Patterns to EBV-Associated Antigens in a Patient With Infectious Mononucleosis. New Eng. J. Med. (in press)

Serial No. NCI - 4808

1. Office of the Associate Scientific Director, Viral Oncology, Etiology
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on the Cellular Immune Defense Mechanisms in Response to Tumor Induction

Previous Serial Number: Same

Principal Investigator: Dr. Harry G. Steinman

Other Investigators: Dr. Arnold K. Fowler
Dr. Alfred Hellman

Cooperating Units: None

Man Years:

Total	1.5
Professional:	.9
Others:	.6

Project Description

Objectives:

The humoral immune mechanisms play a major role in the mammalian defense mechanisms against conventional acute viral infections. In contrast, cellular immune mechanisms appear to be important in the defense against the malignant process. The lymphocyte is a major element of the cellular immunity system. It has been demonstrated that the ability of the lymphocyte to respond immunologically is impaired in such diseases as Chronic Lymphocytic Leukemia and Hodgkins disease.

It is planned to examine factors modifying cellular immunity, particularly the inducing factors, as well as the resultant malignant cell. The nutritional requirements and cell membrane changes associated with such cellular responses will primarily be investigated.

Methods Employed:

It has been recently observed that a significant fraction of the lymphocyte population of immunized hosts undergo blastogenic transformation when stimulated with specific antigens. The capacity of a lymphocyte population to undergo blastogenesis is considered to be a measure of the immunocompetency of the host. Many viruses, including oncogenic viruses, are immuno-suppressive i.e., repress the blastogenic response, whereas others are immuno-enhancing. This response can be quantitated by means of standard radioactive labeling techniques. The blastogenic response, among others, will be utilized and investigated in these studies.

Major Findings:

By a means of a model system, using a non-specific mitogenic stimulant to promote blastogenesis in murine spleen lymphocyte cultures, it has been established that a number of factors, both chemical and physical, are required at critical levels in order for the blastogenic process to occur. It has been noted that the stimulant interacts with both the lymphocyte itself and the serum supplement of the culture medium to such a degree that the effective concentration of the stimulant is reduced considerably and is a function of the concentrations of each of the other two components of the system. This finding permitted the development of a chemically defined medium containing various serum substitutes.

By the elimination of the variable secondary effects introduced by the use of serum, it has been possible to examine the formation of interferon, one of the immunologically active molecules released by stimulated lymphocytes. On correlating the production of interferon with several of the sequential events which occur during the blastogenic cell cycle, it was found that interferon release was associated with the increase in synthesis of RNA.

By means of the chemically defined medium it was found possible to adjust the culture conditions so that the formation of interferon took place under conditions where increased DNA synthesis, a later phase of the cell cycle, failed to occur.

Significance to Biomedical Research and the Program of the Institute:

The effects of the oncogenic process in suppressing the immunologic capacities of the host during the course of neoplasia can perhaps be developed as a diagnostic tool as well as providing basic information and techniques as more is learned about the specific processes of the host defense mechanism. Moreover, supplemental therapeutic regimens may be developed.

Proposed Course:

Studies of the biochemical factors which govern lymphocyte reactivity will be continued in order to develop a fuller understanding of the role of the lymphocyte in the immunologic process. The various stages of the immune response will continue to be examined to determine specific biochemical controls. Studies on the role of the cell membrane in lymphocyte reactivity will be initiated.

Honors and Awards:

None

Publications:

Fowler, A. K., Hellman, A., Steinman, H. G. and Quatrale, A. C.: Studies on the Blastogenic Response of Murine Lymphocytes. I. Quantitative Measurement of Stimulation by Phytohemagglutinin. Proc. Soc. Exp. Bio. Med. 138: 345, 1971

Steinman, H. G., Fowler, A. K. and Hellman, A.: Studies on the Blastogenic Response of Murine Lymphocytes. II. The Effects of Serum-Stimulated-Cell Interactions on Phytohemagglutinin-Induced Stimulation. Proc. Soc. Exp. Bio. Med. (in press)

Hellman, A., Fowler, A. K., Steinman, H. G. and Buzzerd, P. M.: Studies of the Blastogenic Response of Murine Lymphocyte. III. Specific Viral Transformation. Proc. Soc. Exp. Bio. Med. (in press)

Fowler, A. K., Steinman, H. G., Reed, C. D. and Hellman, A.: Studies of the Blastogenic Response of Murine Lymphocyte. IV. Endocrinological Effects. Proc. Soc. Exp. Bio. Med. (in press)

Serial No. NCI - 4809

1. Office of the Associate Scientific Director, Viral Oncology, Etiology
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Characterization of GS Antigen in Mouse Uterus

Previous Serial Number: None

Principal Investigator: Dr. Phyllis Kind

Other Investigators: Dr. Alfred Hellman
Dr. Arnold K. Fowler

Cooperating Units: None

Man Years:

Total	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

A. To determine if the material induced by estrogen in the mouse uterus has both gs-1 and gs-3 antigenic determinants.

B. To isolate and characterize the gs antigen in the estrogen stimulated mouse uterus.

Methods Employed:

A. Immuno diffusion and complement fixation have been used to detect the antigen and to investigate its cross reactivity with viral antigens.

B. A variety of animal species are being immunized to obtain antisera capable of detecting gs antigenic determinants. GS antigens are being extracted from stimulated mouse uterus using the methods that have been reported for extraction of viral gs antigen.

Major Findings:

A. Analysis of stimulated uterus by immuno diffusion has yielded clear evidence of gs-3 activity. Determination of the presence or absence of gs-1 activity awaits further analysis.

B. Gs antigenic activity has been found to be localized at the cathodal portion of polyacrylamide gel electrophoresises. It is not yet possible to associate the antigenic activity with a single band.

Significance to Biomedical Research and the Program of the Institute:

Characterization of the gs antigenic materials will allow further studies of the control of their expression as well as studies of the role of gs antigens in the induction and maintenance of the malignant state.

Proposed Course:

Major efforts will be directed toward isolation of gs antigens from mouse uterus and toward development of more sensitive and accurate methods for measuring these antigens.

Honors and Awards:

None

Publications:

None

SUMMARY REPORT

The Office of Program Analysis and Communications (PAC) continued its major roles in the management of SVCP data and information, analytical statistical assistance in research projects, and scientific information storage, retrieval, and distribution. Some highlights are as follows:

Statistical and Data Services

Offered to all researchers in the SVCP is a comprehensive statistical service (consultation on problems of research design, data procurement, data management, and statistical analysis of survey or experimental findings). A completely automated data processing routine directed by experienced technicians maintains direct access to the main NIH computer. Consultation and planning service is also given to SVCP contractors in developing local automation systems for managing and processing experimental data.

Automated Inventories of SVCP Resources

1. SVCP Serum Bank: Maintaining a computerized inventory of serum specimens held in the Bethesda area, PAC manages the continuous data input, updating, and distribution of specimens for testing to program scientists, from repositories containing around 40,000 serum specimen units.

2. Local Inventories: Plans and installs complete institutional automated specimen inventories in laboratories collaborating in SVCP research.

3. Central PAC Inventory: PAC is promoting compatible automated systems and codes for specimen inventories in all institutions participating in the SVCP. This includes the capture and automation of complete clinical, demographic, and laboratory information on specimen donors. Goal is a central specimen inventory at NIH making available for all of SVCP the many large specimen repositories throughout the United States.

Progress Reports

A system of managing regular (triannual and annual) progress reports from all SVCP research contracts is maintained. In addition to the logistics of monitoring and procurement, PAC compiles and distributes to program scientists condensed summaries of the actual progress reports.

Scientific Information Management

The Information Unit continued to focus attention on scientific information retrieval in the area of viral oncology, and its dissemination to program scientists. Sources of information are scientific journals, books, and other notifications and summaries of current research in the field. Major contributions of the Information Unit in FY 1972 were as follows:

1. Bibliographic Service: A semi-automated system is maintained for storage, rapid search, identification, and reference print-outs covering almost any desired topic in the published literature on viral oncology.

2. Viral Tumorigenesis Report: This semi-annual publication contains indexed summaries of current pertinent research projects, thereby presenting a panoramic view of viral oncology research.

3. Report on NCI Support of Cancer Virology Grants: This quarterly report presents an organized summary of updated fiscal data on current NCI research grants in the cancer virology area and has limited distribution to NCI adminstrators. The basic information is received from the Program Analysis and Reports Section of NCI.

4. Viral Oncology Contractor Directory: This quarterly publication contains the names, addresses, and telephone numbers of contractors, principal investigators, project officers, and contract specialists within the Viral Oncology Program. Its purpose is to facilitate and expedite communications between staff members and contractors.

5. Compilation of Journal Instructions to Authors: This displays in one volume the instructions-to-authors from a majority of pertient scientific journals. It is a reference aid for research investigators in writing papers and also for the secretaries who type them. The compilation is updated and expanded periodically.

Other responsibilities of the Information Unit are: administration of library facility; collection and distribution of translations of foreign publications in viral oncology; maintainence and lending of recorded tapes on NIH seminars related to viral oncology; and continuous compilation of the SVCP bibliography containing citations to all papers published by Viral Oncology staff members and research contractors.

Summary Report

Office of the Coordinator for Ultrastructural Studies

The Virus Studies Section continues to study different aspects of the relationship between oncogenic viruses and their host cells:

a) the search for viruses in different tumors by electron microscopic examination,

b) the examination of the life cycle of oncogenic viruses of the herpes type in their host cells,

c) the study of early events in viral infection which may result in "neoplastic transformation," and

d) the study of cellular organelles in relation to the reproduction of RNA-containing viruses.

Dr. Dalton in collaboration with Dr. Szakacs, St. Joseph's Hospital, Tampa, Florida, established 15 tissue cultures from human biopsies obtained from solid tumors. Ten cell lines are fibroblastic or epithelial in appearance, and five are growing as suspension cultures containing lymphoid blast cells resembling those blasts obtained by cultivating Burkitt's lymphomas. In the majority of these lines a series of cell types from relatively undifferentiated lymphoblasts to practically mature plasma cells have been observed. Except in one instance these cells are not the original tumor cells but are thought to be "normal" cells which have developed into continuous lines as a result of the stimulus from the presence of E B virus. These cultures were screened for the presence of virus particles. In four out of five cell lines Epstein-Barr virus was found as shown by morphological examination and complement fixation tests. About 3-5% of the cells contain in their cytoplasm an aberration of the endoplasmic reticulum, known as tubulo-reticular array. It was suggested previously that these formations may represent RNA precursors of certain myxo- and paramyxoviruses. Dr. Heine in collaboration with Dr. Schaff discovered, by using ultracytochemical methods, the sensitivity of these structures to proteases. These results make it clear that the structures are not direct RNA precursors to RNA-containing viruses.

Dr. Heine in collaboration with Dr. Hinze, University of Wisconsin, Madison, Wisconsin, studied the life cycle of H. sylvilagus in rabbit kidney cells. This DNA-containing oncogenic virus of the herpes type develops in infected cells and provokes changes in these cells similar to those described previously for H. saimiri. Both viruses produce a series of changes in their host cells known to be characteristic for cytomegaloviruses, i.e., the formation of nuclear and cytoplasmic inclusion bodies. However, in contrast to the classical cytomegaloviruses the intracellular maturation of the virions under study appears to be quite unique resulting in two morphologically different types of mature virions. These results initiated a detailed comparative study between tumor producing and non-oncogenic viruses of the herpes type and their relation to the host cell. We hope

to elucidate further similarities and differences between both kinds of viruses.

Several studies which are concerned with early events in viral infection are under way in this laboratory.

Dr. Bader in cooperation with Dr. Boone, Cell Biology Section, VBB, NCI, investigated the mode of entry of RNA-containing tumor viruses into cells by using isolated membrane vesicles as a model system. The methods of membrane isolation have not proven adequate thus far to answer the questions, and new procedures for isolating intact surface membranes are under investigation.

Dr. Suskind continued to study the intracellular mechanism of infection with Rous sarcoma virus during the eclipse phase, especially events which occur within the nucleus, by quantitative light and electron microscopic autoradiographic techniques. He succeeded in showing differences in both morphology and function between nucleoli of RSV-transformed, newly infected and uninfected cells with respect to the rate of their recovery from the effects of low doses of Actinomycin D. The results suggest the formation of new binding sites of Actinomycin D to specific DNA sites in nucleoli of transformed and newly infected cells. These experiments will be expanded using non-transforming, Rous-associated virus and thermo-sensitive mutants as well as protein and polysaccharide precursors.

Dr. Heine in collaboration with Dr. Beaudreau, Oregon State University, Corvallis, Oregon (Contract #NIH-NCI-71-2175) initiated a study to examine the fate of virion RNA after infection. In order to study the fate of viral RNA in infected cells purified MC-29-virus was prepared containing H^3-uridine at high specific activity ($1\text{-}2 \times 10^5$ cpm/ug RNA). Cells infected with virus thus labeled have been examined 1-8 hrs. after infection and the label was recovered from a polysome fraction. Fractions that contain label will be examined in the electron microscope to confirm the presence of polysomes.

Dr. Bader studied the inhibition of mitochondrial RNA polymerase in mammalian and avian cells by ethidium bromide and observed morphological alterations by electron microscopy in mitochondria of chick embryo, rat embryo and mouse spleen and thymus cells (JLS-V5) after exposure to the drug. The effects of ethidium bromide were reversible; after its removal the cells returned to normal within a few days with respect to their morphology and growth characteristics. Chick embryo cells which had been previously treated with ethidium bromide were infected with Rous sarcoma virus and were then examined for transformation and virus production. Drug-treated cells became transformed and produced virus as seen by electron microscopy. The results of these experiments show clearly that the mitochondrial DNA template is not involved in the synthesis of Rous sarcoma virus.

Dr. Dalton has studied the details of the replication of Type B (mouse mammary tumor virus) and type C (avian, murine and feline leukemia-sarcoma viruses) particles. The results of these high resolution electron

microscopical studies demonstrate that newly formed particles have a much more precise morphology than older particles. They also indicate that the type C particles so far studied have a morphology characteristic for the species in which they are indigenous. When chrome osmium is used as a fixative, murine type C particles at the budding stage possess a relatively thick (100 Å) and distinct outer component of the developing nucleoid whether the virus is replicating in mouse or human cells. In budding feline type C particles this layer is thinner (50 Å) but still distinct. Type C virions growing from ESP-I (human) cells do not exhibit a clearly separate layer while type C virions growing from RD-114 cells have a distinct thin layer similar to the feline type C virions.

Supporting Units:

a. The tissue culture unit (Mrs. J. Kondratick, Mrs. T. Ben) established 15 new cell lines from human biopsies. Chick embryo cells, rat cells and JLS-V5 cells were maintained to provide material for membrane studies, autoradiographic examination and drug evaluation. Chick myeloblast cultures infected with BAI strain A virus were maintained for virus studies. High titer pools of Rous sarcoma virus and RAV-50 were prepared.

b. The photography unit (Mr. D. Jones) has printed and enlarged all electron micrographs taken at 4 electron microscopes in the Virus Study Section. These are appr. 2250 routine photographs, 800 lantern slides and 25 exhibition prints. Three hundred prints were prepared for publication. In addition, special photographic work was done for the Office of the Associate Scientific Director for Viral Oncology (Dr. Moloney), VLLB (Dr. Ablashi), Georgetown University (Dr. Stewart) and VBB (Dr. Chirigos).

c. In the animal room (Mr. Shoemaker) 13 spontaneous tumors are being carried in in vivo transplants and about 1000 mice of different inbred strains are bred to carry these transplants.

d. The electron microscope preparation unit (Messrs. B. Elliott, Jr., R. Moore, R. Rusten and Mrs. M. Bigelow) received approximately 600 specimens for processing for electron microscopy. This material was obtained from the staff of the section, and from collaborating units (M. D. Anderson Hospital and Tumor Institute, Houston, Texas, St. Joseph's Hospital, Tampa, Florida, and University of Wisconsin, Madison, Wisconsin). Ultrathin sections were cut from approximately 450 specimens. Special preparative methods for ultracytochemical investigations were developed, and autoradiographic techniques at the light and electron microscope level were used as routine methods in addition to conventional methods used in electron microscopic studies.

Number of visitors from foreign countries: 1; number of invited presentations as lectures or seminars: 7.

Dr. Dalton: Invited speaker EMSA, August, 1971. "DNA viruses associated with neoplasia."

Publications:

Further analysis of the detailed structure of type B and C particles. J. Nat. Cancer Inst., April, 1972.

Observations on the details of ultrastructure of a series of type C viruses. Cancer Research, June, 1972.

Contributor and co-editor of "Ultrastructure of animal viruses and bacteriophages - An Atlas" which is now in the hands of the publishers.

Chaired session on "Properties of oncogenic viruses" at M. D. Anderson Hospital and Tumor Institute symposium entitled: Molecular Studies in Viral Neoplasia, March 8-10, 1972.

As a member of the NIH Florence Agreement Committee, reviewed over 100 applications for duty-free entry of transmission and scanning electron microscopes.

Serial No. NCI-4812
1. Office of the Associate Scientific Director for Viral Oncology, Etiology
2. Ultrastructural Studies Unit
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on Virus-host Cell Interactions.

Previous Serial Number: None.

Principal Investigator: Dr. Ursula Heine

Other Investigators:
Dr. D. V. Ablashi
Dr. G. S. Beaudreau
Dr. A. J. Dalton
Dr. F. Haguenau
Dr. H. H. Hinze
Dr. G. R. Krueger
Dr. Z. Schaff
Dr. J. Szakacs

Cooperating Units: Microbiology Section, VBB, NCI
Hematopathology Section, Laboratory of Pathology, NCI

Cooperating Units (outside NIH):
St. Joseph's Hospital, Tampa, Florida
University of Wisconsin, Medical School, Madison, Wisconsin
Oregon State University, Dept. of Agricultural Chemistry, Corvallis, Oregon
Laboratoire de Médecine experimentale, Collège de France, Paris.

Man Years:

TOTAL:	3
PROFESSIONAL:	2
OTHER:	1

Project Description

Objectives:

A). To study and identify selected viruses in relation to their host cells by ultrastructural and other biological methods.

B). To study the relationship between structure and function of cellular components before and after infection with tumor viruses.

Methods Employed:

Virologic methods and electron microscopic techniques, including ultracytochemistry, were employed for cell studies at the ultrastructural level. Variations in cellular functions were investigated by biochemical methods (radioisotope tracers, cell fractionations, RNA extractions gradient sedimentations and spectrophotometry), in addition E M techniques were used.

Major Findings:

A. 1) Tissue culture isolates from solid human tumors have been studied electron microscopically to determine the presence or absence of viruses in the isolates. To date we have established eight cell lines of lymphoblastoid cells. In five cell lines the Epstein-Barr virus (EBV) is present as shown by morphological examination and complement fixation tests. One cell line contains both EBV and a cytomegalovirus, and cells of another line contain only a cytomegalovirus genome. In the cytoplasm of about 3-5% of the lymphoid blast cells tubulo-reticular structures, a modification of the endoplasmic reticulum, have been observed; and it was suggested that these structures may represent RNA precursors of certain RNA viruses (Gyorkey, Sinkovics, and Gyorkey; Lombard, Cabanie, and Izard). It is of importance to obtain further information concerning the composition of these structures, which - in turn - may lead to a better understanding of their role in the cell. Digestion studies have been carried out on ultrathin sections using different enzymes, and the inclusions have been found to be extremely sensitive to pronase and pepsin. They are not attacked by RNase. Likewise, special embedding and staining procedures to demonstrate the presence of RNA or DNA containing structures in the cell at high resolution gave negative results when applied to the tubulo-reticular arrays. The results of our investigations make it clear that the tubulo-reticular structures are mainly composed of proteins and are not direct precursors of RNA containing viruses. This work was done in collaboration with Dr. Dalton and Schaff. A publication is in preparation.

2) In a continuing study we examined the relationship of different herpesviruses to their host cells. In collaboration with Dr. Ablashi the propagation of H. saimiri in a human cell line (WI-38) was achieved. Cytopathic effects were produced when cells were pretreated with diethylaminoethyl-dextran. The presence of complement-fixation antigen in infected cells was used as a determinant of the viral infection. Virions in different stages of maturation have been observed in the infected cells by electron microscopy. Inoculation of H. saimiri into owl monkeys resulted in the development of malignant lymphomas with

lymphocytic leukemias. The virus was recovered from the monkeys and identified as H. saimiri. However, virus particles could not be detected in the tumors and different organs of the diseased monkeys.

In collaboration with Dr. Hinze the life cycle of H. sylvilagus, known to be indigenous in the cottontail rabbit, was studied in rabbit kidney cells grown in vitro. Electron microscopic examination of foci of infected cells revealed early stages of virus development in the nuclei. Immature virions are similar in their morphology to known herpesviruses. The envelopment of the naked particles occurs either at nuclear or at preformed cytoplasmic membranes giving rise to two kinds of mature virions characterized by morphologically different coats. Cellular response to the infection is expressed by the appearance of nuclear inclusion bodies and a few lysomes in the cytoplasm.

3). In collaboration with Dr. Krueger the induction of malignant lymphomas in mice by interference with the immune response against a variety of antigens was demonstrated. It appears that this lymphoma development is less dependent upon the oncogenic transformation of cells by viruses but is rather a consequence of simultaneous persistent antigenic stimulation and immunosuppression. Since the morphology of human lymphomas is well described, yet the initiating lesions are still poorly known, a systematic morphologic investigation was carried out on the development of immunologically induced malignant lymphomas in mice as background for initiating a careful search for comparative lesions in human pathology. Cells of well established tumor showed only slight differences from normal lymphoblasts such as an increased esterase activity and the presence of cytoplasmic annulate lamellae. Virus particles of the C-type were identified by electron microscopy only in early tissue culture passages of the BALB/c lymphoma but not in the DBA lymphoma. This project is completed.

B. In collaboration with Dr. Beaudreau (Contract NCI-71-2175) a study is initiated to examine the fate of virion RNA after infection. It is apparent that two events are important steps in the infection and transformation process: a) the cell translation of the viral genome and b) the formation of the virus-specific DNA polymerase. The DNA polymerase assay has proven to be as sensitive as the focus-forming assay for detecting low quantities of virus in culture fluids. Culture fluids from established human lymphoid cultures (T-13) have been tested, and it was found that these fluids have considerable DNA polymerase activity. Electron microscopic examinations of samples from the gradient fractionation have not yet revealed any known virus particles.

Significance to Biomedical Research and the Program of the Institute:

A. 1. The detection of viruses in human tumors as a causative agent for unlimited growth is of importance for the etiology of human cancer. The study and characterization of unknown cellular structures regularly present in isolates from solid human tumors containing a virus or viral genome is decisive for the better understanding of metabolic events in these cells.

2. Cells infected with H. saimiri or H. sylvilagus are an excellent model for the study of human cancer, since both viruses are not detectable in vivo but readily rescued in vitro.

3. The systematic morphologic investigation of the development of malignant lymphomas in mice establishes a background for initiating a search for comparative lesions in human pathology.

B. RNA viruses are known to be causative agents for some malignant growths. The examination of cellular changes early after infection is important for the understanding of the infectious process. It is the goal of this project to relate biochemical events to ultrastructural changes in the cells.

Proposed Course:

The study on oncogenic herpes type viruses in relation to their host cells will be expanded and the results will be compared with the behavior of nononcogenic herpes viruses. It is hoped to establish a basis for a new systematic order of these viruses.

Early events in cells infected with RNA viruses shall be investigated further to determine physical and biochemical differences to uninfected cells. We shall try to characterize cell fractions containing the virus RNA in the early infection period.

Honors and Awards:

Dr. Heine was a Fogarty Fellow under the Franco-American Scientific Exchange and worked from Sept. 6 to Nov. 30, 1971, in the Laboratoire de Médecine experimentale, Collège de France, Paris.

Dr. Heine presented the following papers:

"The influence of toyocamycin on mammalian and avian cells grown in vitro", Sept. 29, 1971, 2nd meeting of the European Nucleolar Workshop, Lake Balaton, Hungary.

"Nucleolar changes in HeLa cells exposed to the antibiotic toyocamycin", Oct. 17, 1971, Laboratoire de Biologie Cellulaire, Faculté des Sciences, Orsay, France.

"Herpesviruses and their relation to the infected cell, an electron microscopic study", Nov. 29, 1971, Institute of Experimental Cancer Research, Heidelberg, Germany.

"Ultrastructural analysis of herpes type viruses", March 8, 1972, 25th Ann. Symposium on Fundamental Cancer Research, Houston, Texas.

Dr. Heine was invited to be session chairman, "Action of biological and physical agents" at the 2nd meeting of the European Nucleolar Workshop, Lake Balaton, Hungary, and participated there at two roundtable discussion: 1) "Nucleolar metabolism and fine structure", 2) "Experimentally induced changes of nucleolar fine structure and function."

Publications:

Heine, U. I., Ablashi, D.V., and Armstrong, G. R.: Morphological studies on Herpesvirus saimiri in subhuman and human cell cultures. Cancer Res. 31: 1019-1029, 1971.

Ablashi, D.V., Armstrong, G. R., Heine, U., and Manaker, R. A.: Propagation of Herpesvirus saimiri in human cells. J. Nat. Cancer Inst. 47: 241-244, 1971.

Ablashi, D. V., Loeb, W. F., Valerio, M. C., Adamson, R. H., Armstrong, G. R., Bennett, D. G., and Heine, U.: Malignant lymphoma with lymphocytic leukemia induced in owl monkeys by Herpesvirus saimiri. J. Nat. Cancer Inst. 47: 837-855, 1971.

Krueger, G. R., and Heine, U. I.: Morphogenesis of two immunologically induced mouse lymphomas. Cancer Res., in press.

Heine, U., and Hinze, H. C.: Morphological studies on Herpesvirus sylvilagus in rabbit kidney cell cultures. Cancer Res., in press.

Serial No. NCI-4814

1. Office of the Associate Scientific Director for Viral Oncology, Etiology
2. Ultrastructural Studies Unit
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Autoradiographic and Ultrastructural Studies of the Nucleus of Chicken Fibroblasts during the Eclipse Phase of Infection with Rous Sarcoma Virus.

Previous Serial Number: None.

Principal Investigator: Dr. R. Gerald Suskind.

Other Investigators: (outside NIH)
Dr. Francoise Haguenau
Dr. Giancarlo Rabotti
Dr. Susan C. Michelson-Fiske

Cooperating Units: Laboratoire de Medecine Experimentale, College de France, Paris.

Man Years:

TOTAL:	2
PROFESSIONAL:	1
OTHER:	1

Project Description

Objectives:

The intracellular mechanism of infections with Rous Sarcoma Virus during the eclipse phase of the events within the nucleus leading to "neoplastic" transformation is under investigation using quantitative light and electron microscopic autoradiographic techniques.

Methods Employed:

Tissue culture of chick fibroblasts and selection of embryos susceptible to total transformation by the SR strain of RSV; RIF testing; electron microscopic screening for C particles or mycoplasma; cloning and plaque purification and titration of virus pools; interference titration of RAV. Quantitative autoradiographic techniques at the level of the

light and electron microscope using tritiated RNA and DNA precursors and tritiated Actinomycin D with high resolution physical development; ultrastructural examination.

Major Findings:

Differences in both morphology and function between nucleoli of RSV-transformed cells, newly-infected cells and uninfected cells with respect to the rate of their recovery from the effects of low doses of Actinomycin D have been observed. It has been shown that both the recovery of RNA synthesis in the fibrillar portion of the nucleolus, as well as the restitution of nucleolar morphology occurs more rapidly in infected cells than in uninfected cells. Furthermore, this recovery occurs at parallel rates in transformed cells and cells newly infected with RSV, but at a slower, divergent rate in uninfected control cells. It could be shown in this manner that nucleoli of infected cells develop a reduced sensitivity to Actinomycin within about 80 minutes of infection. In the hope of measuring a parameter of the viral genome for transformation and its cellular sites of attachment attempts have been made to explore the mechanism of this phenomenon. Quantitative autoradiographic experiments at the level of the light microscope have indicated a concomitant increase in the relative binding of tritiated Actinomycin to nucleoli of transformed and newly infected cells at the time of increased RNA synthesis. The formation of new binding sites of Actinomycin to specific DNA sites in nucleoli are suggested by these results. Attempts to confirm these observations with high-resolution autoradiography have so far been only partially successful. Whereas the labeling of nuclei and nucleoli with tritiated Actinomycin could be established with high concentrations of AD after exposure time of 5 months only occasional labeled nuclei were found at low concentrations, so far only in transformed cells. However, quantitative assays of the relative distribution of the label has not been achieved because of the paucity of the label. Other experiments on the sequence and distribution of DNA label have shown an identical delayed inhibition of nuclear DNA synthesis by Actinomycin in newly-infected and in uninfected cells. However, in transformed cells heavy cytoplasmic labeling unaffected by Actinomycin, was observed after a twenty-minute pulse label. This is localized ultrastructurally near the plasma membrane(and has also been noted in association with virus particles). The presence of a latent mycoplasma infection, however, precludes any inference as to the viral nature of this DNA until further results are obtained. The relevance of these phenomena to the development of the transformed state experiments using non-transforming, Rous-associated virus and thermo-sensitive mutants as well as protein and polysaccharide precursors is under study.

Significance to Biomedical Research and the Program of the Institute:

Technical methods for studying morphologic and functional changes within the nucleus during early stages of infection with Rous Sarcoma Virus by means of high resolution autoradiography and quantitative light microscopic autoradiography have been developed and results relevant to the induction of the transformed state have been obtained thereby. The development of lower sensitivity to Actinomycin D within 80 minutes of infection has been found and related to changes in the binding of Actinomycin to nucleoli. Virus-induced alterations in specific DNA sites are suggested thereby.

Proposed Course:

It is proposed to study further the sequence and distribution of DNA synthesis in the transformed cell system in relation to these observations and the effect of non-transforming viruses and/or thermosensitivie mutants using also protein and polysaccharide precursors.

Honors and Awards:

At the invitation of the Chairman of the Scientific Program, Dr. Suskind presented a paper entitled: "Autoradiographic and Ultrastructural Studies of the Nucleolus of Chicken Fibroblasts during the Eclipse Phase of Infection with Rous Sarcoma Virus" at the 2nd European Nucleolar Workshop, Lake Balaton, Hungary, on September 27.

Publications: None.

Serial No. NCI-4817
1. Office of the Associate Director for Viral Oncology, Etiology
2. Ultrastructural Studies Unit
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: (A) A study of the degenerative changes in mitochondria induced by ethidium bromide.

(B) Effect of ethidium bromide on Rous sarcoma virus and Rauscher leukemia virus production.

(C) Adsorption and penetration of RNA-containing tumor viruses into CE cells.

Previous Serial Number: NCI-4813.

Principal Investigator: Dr. Artrice V. Bader

Other investigators: (Project C) Dr. Charles Boone

Cooperating Units: Cell Biology Section, Viral Biology Branch, NCI

Man Years:

TOTAL:	2
PROFESSIONAL:	1
OTHER:	1

Project Description

(A). To determine if inhibition of mitochondrial RNA polymerase in mammalian and avian cells by ethidium bromide would be reflected in mitochondrial changes which could be observed by electron microscopy.

To establish a model system for morphological changes so that virus production in avian and mammalian cells could be studied.

(B). To determine the role of the mitochondria in the reproduction of RNA-containing tumor viruses.

(C). To investigate the mode of entry of RNA-containing tumor viruses into cells using isolated membrane vesicles as a model system.

Methods Employed:

(A). Electron microscopy, cell culture.

(B). Electron microscopy, cell culture.

(C). Electron microscopy, phase microscopy, cell culture, differential centrifugation, density layering.

Major Findings:

(A). Morphological alterations were observed in the mitochondria of chick embryo (CE), rat embryo (RE) and a chronically infected continuous cell line of mouse spleen and thymus cells (JLS-V5) after exposure to ethidium bromide. Distinct rearrangement of the mitochondrial cristae (infoldings of the inner mitochondrial membrane) were observed as early as 48 hrs. after exposure to the drug. The mitochondrial cristae become arranged into aberrant configurations and the longer the cells are exposed to the drug, the greater the degenerative effect. Concomitant with the rearrangement of the cristae, the mitochondrial matrix loses its density and mitochondrial granules are also lost. Size variation is a common feature of the mitochondria. With continued treatment, the JLS-V5 cultures eventually became resistant to the drug and normal looking mitochondria reappeared in these cells. Unlike the CE and RE cells, the JLS-V5 cells showed distinct morphological differences at the light microscope level and also had plating properties different from control cells.

The effects of ethidium bromide were reversible; after removal of the drug growth characteristics of the cells returned to normal within a few days and the morphology of mitochondria became similar to those of non-treated cells. There was a variability in stability of ethidium bromide treated CE cultures, limiting experiments with these cells to one week. RE cells were eliminated from further experimental studies because of their high frequency of spontaneous transformation which occurred in both treated and non-treated cultures. The results of this work established a model system for studying the role of mitochondria in the production of RNA-containing tumor viruses.

(B). Ethidium bromide inhibits mitochondrial RNA polymerase and produces defective mitochondria which can be observed with the electron microscope. CE cells which had been previously treated with ethidium bromide were infected with Rous sarcoma virus and were then examined for transformation and virus production. Virus production was ascertained by focus assay of cell culture fluids and observation of budding particles by electron microscopy. Ethidium bromide treated cells became transformed and produced virus particles in a manner similar to infected control CE cells which had never been exposed to ethidium bromide. Some inhibition in the amount of transformation and virus production was found in the

treated cultures but this could be attributed to other physiological effects of ethidium bromide on cells. Virus particles were observed budding from cells which contained defective mitochondria. The virions found in the extracellular spaces of treated and non-treated cultures had identical morphology.

(C). Membrane vesicles were isolated from CE cells by differential centrifugation and isopycnic banding. This project was done in collaboration with Dr. Charles Boone. CE cells were exposed to a mixture of Rous sarcoma virus and Rous associated virus prior to isolation of plasma membranes. Membrane pellets obtained by differential centrifugation were fixed for electron microscopy. Membrane vesicles were formed with this procedure but there was difficulty in obtaining consistent purity of vesicles. Because of insufficient attachment of virions to these vesicles, it was not possible to make a definite judgment about the mechanism of entry. It was hoped that the use of membrane vesicles would eliminate the quantitative insufficiency which was encountered in earlier studies when intact cells were used to investigate the mode of entry of these viruses. As to date, the method of membrane isolation, according to our present procedures has not proven adequate to answer this important question.

Proposed Course:

(A and B). The ethidium bromide system has exciting potential for studies of parameters of tumorigenesis. The fact that cells can continue to grow in the presence of defective mitochondria suggesting an inhibition or loss of oxidative metabolism (Kreb's cycle) is the focus point for future projects. This system will be used to examine metabolic factors effecting tumorigenesis.

(C). New procedures for isolating intact surface membranes are under investigation. Also other approaches to utilizing the intact cell for viral entry experiments will be applied.

Significance to Biomedical Research and this Institute:

(A and B). The results of these experiments demonstrate unequivocally that mitochondria play a relatively minor role,if any, in the reproduction of oncornaviruses. This conclusion contrasts directly with previous reports (Mach and Kara, 1971; Kara, Mach and Cerna, 1971) that state that Rous sarcoma virus is replicated in the mitochondria of cells. The results of this study show that the mitochondrial DNA template is not involved in the synthesis of Rous sarcoma virus and that virions are produced in cells which have been exposed to ethidium bromide and reveal defective mitochondria. The reproduction of Rous sarcoma virus and Rauscher leukemia virus as representatives of RNA-containing tumor

viruses is important to the understanding of the infectious cycle and the role of these viruses in oncogenesis. Also it is significant that mammalian and avian cells are able to grow with a deficiency or loss in energy provided by the Kreb's cycle. This system offers an attractive model for the study of the role of mitochondria in tumorigenesis.

(C). Several hypotheses have been proposed for the mode of entry of RNA-containing tumor viruses into cells but the problem remains unresolved. The method of virus entry into the cell is important to an understanding of the infectious cycle of RNA-containing tumor viruses and their role in oncogenesis.

Honors and Awards: Invited to be session chairman, RNA tumor viruses, American Society for Microbiology, Annual Meeting, Phila., Pa., April 23-28, 1972.

Publications:

Bader, A. V., and Steinberg, R. L.: A photographic developing unit for use in autoradiographic electron microscopy. Stain Technology 47: July , 1972.

Orr, H. C., Harris, Jr., L. E., Bader, A. V., Kirschstein, R. L., and Probst, P. G.: Cultivation of cells from a fibroma in a rattlesnake, Crotalus horridus. J. Nat. Cancer Inst. 48: 259-264, 1972.

SUMMARY REPORT

Office of Program Resources and Logistics

The Office of Program Resources and Logistics within the Office of the Associate Scientific Director for Viral Oncology is responsible for the review and scientific management of collaborative research contracts providing resources and logistical support to the Special Virus Cancer Program and the Viral Oncology Area. This Office was established during this fiscal year to centralize the scientific administration and management of research resources and logistical functions and to unify these activities within the Office of the Associate Scientific Director. In this way individuals responsible for coordinating these support activities could provide to the entire Program with an awareness of the overall scope of activities. This would avoid any special consideration to a particular area, any unnecessary duplication of effort, and the appearance of any undesirable competition within the Program.

Many of the research investigations carried out in the Viral Oncology Area depend on the availability of clinical and laboratory materials of optimal purity, viability, and potency. Comparable studies in an integrated program of international scope, as encompassed in the SVCP, make more meaningful and rapid progress when adequate quantities of standardized reagents, cell cultures, and test animals are available. The Office of Program Resources and Logistics provides these supportive activities through various contract operations as detailed below.

To support this office and its activities, a new contract review body was established during this year, the Viral Oncology Program Resources & Logistics Advisory Group. This Group was established by the Associate Scientific Director and constitutes a standing committee to provide support and make recommendations concerning resources and logistics matters, and to conduct appropriate reviews for those contracts within the R & L area of Program. The group is chaired by the Chief, Office of Program Resources & Logistics and is responsible to the Office. The membership includes representatives from the three Branches and the major areas of Viral Oncology.

Because of organizational changes in administrative management of collaborative research contracts, a variety of contracts formally within the R & L Segment and other segments within SVCP became the responsibility of the new Office of Program Resources & Logistics. These contracts represent three general areas of activities and include the following:

1. Activities directed toward production of virus and viral reagents, virus monitoring, and characterization.

2. Contracts concerned with acquisition, collection, storage, inventory, and distribution of human resource specimen material.

3. Contracts concerned with animal resources, including production of pathogen-free and germ-free species of animals, breeding of primates, maintenance of animal colonies, and containment-type holding facilities.

Another activity of the OPRL is the preparation of an annual catalog listing and describing the research resources available to collaborating laboratories within the Program. Usually the information provided for each item includes origin, processing procedure, degree of purity, and infectivity titer or other measures of biological activity. Additionally, in collaboration with the Office of Program Analysis and Communication, the OPRL is concerned with the development of a computerized central inventory for the sera, tumor tissue, cell outgrowths, and other human specimen materials continuously being acquired by the Program. The central inventory will greatly facilitate matching investigators requests for human materials with specimens available, regardless of the geographic location of the repository or laboratory at which it is stored.

During this past year in anticipation of an expected increase of the overall Program in the future and the limitation of personnel available, consideration was given to the establishment of a prime contract as a mechanism for dealing with resources and logistical support for Program. With this in mind, an advertisement was published in the Commerce Business Daily on November 8, 1971 requesting resumes of experience and capabilities. An Ad Hoc review committee under the chairmanship of the Chief, OPRL was established to review and evaluate the responses obtained. This Ad Hoc group questioned whether, in view of the diversified and specialized elements under the project contract, any of the respondents had demonstrated requisite qualifications. Additionally, the Ad Hoc group recommended a re-evaluation of project needs and suggested that issuance of a request for proposal be deferred.

As a result of these decisions the OPRL will continue to be involved in not only the general management of the resources program, but also in those daily activities required to make available to collaborating investigators necessary research resources.

Within the Office of the Chief, support is provided by two staff scientists and a secretary who assist in responsibilities for the management of the extramural contract operation.

SUMMARY REPORT

VIRAL LEUKEMIA AND LYMPHOMA BRANCH
July 1, 1971 - June 30, 1972

The Viral Leukemia and Lymphoma Branch conducts research designed to elucidate the role of viruses in the etiology of human neoplasms, particularly leukemias, lymphomas and sarcomas. A variety of scientific approaches are used which provide a broad base of knowledge applicable to the identification and isolation of human oncogenic agents and the prevention or control of the disease as it occurs in man. More specifically, the Branch encompasses a range of scientific disciplines including molecular biology, genetics, immunology, biochemistry, pathology and cell culture techniques. In the past year, the emphasis has been away from model systems to the more direct study of human materials.

The Section of Molecular Biology seeks to obtain comprehensive knowledge of the biology and biochemistry of sarcoma and leukemia viruses and conducts quantitative studies on the interaction of oncogenic viruses and cells to determine the mechanisms of viral replication and cellular transformation at the molecular level. The Section of Viral Pathology exerts a multidisciplinary approach towards the in vivo and in vitro study of viral oncogenesis. The areas of study include virology, immunology, pathogenesis and the interferon system, and are pursued emphasizing several viral induced and spontaneous leukemias and sarcomas. The Section of Immunology examines the antigenic nature of oncogenic viruses and the induced tumors as well as the immune response of the host to viral infection and tumor development. The Section of Tumor Viruses is concerned with defining in detail the biological and biochemical properties of tumor viruses in order to understand how they may be applied to the search for human tumor viruses. A "helper" assay to "rescue" oncogenic virus information is currently being applied to human cell systems. The Section of Genetics is concerned with genetic factors of both the tumor virus and the host it infects that are involved in the oncogenic process. Particular emphasis is placed on the viral genes involved in oncogenesis and cellular "susceptibility" genes, particularly those genes of man that predispose individuals to the development of cancer. The Office of the Chief coordinates the research of the various sections by recognizing the scientific freedom of the individual investigators. The office is responsible for establishing collaborative efforts with investigators in other areas of NIH and elsewhere such that information derived from studies within the Branch is constantly being applied in investigations leading to a better understanding of the etiology of human neoplasia.

Potential RNA containing tumor viruses have been recognized by a number of methods based on biological, biochemical, and immunological properties. More recently the reverse transcriptase has provided another potentially extremely sensitive method for virus detection.

The discovery that certain RNA tumor viruses have an enzyme capable of transcribing the viral RNA back into DNA has lead to the possibility of using extremely sensitive biochemical probes to search for evidence of viral etiology of cancers, and especially, cancers in man. Some of the potential applications to the etiology and control of human cancers are:

1. The use of synthetic DNAs produced from the viral RNA to search for complementary RNA in human tumors by DNA-RNA hybridization techniques.

2. The use of highly effective synthetic templates and optimal enzymatic conditions to search for viral reverse transcriptase in human tumor cells.

3. The use of specific antiserum prepared against the purified viral enzymes to identify individuals that have been exposed to the viral enzyme. It is reasonable to expect that the antibodies to viral specific proteins may persist for much longer periods than the virus itself would persist.

Each of the above approaches are being actively followed by members of the Viral Leukemia and Lymphoma Branch.

Following the initial reports of RNA-dependent DNA polymerase in the virions of certain RNA tumor viruses, it was important to see if the enzyme was specifically restricted to tumor viruses and whether it was specifically restricted to tumor cells. All of the oncogenic RNA viruses tested so far have been found to have DNA polymerase, as indicated both by endogenous reaction using the viral RNA and by synthetic polymer-stimulated reactions, using such templates as poly rA.rU, poly rI.rC and poly rA.dT. The non-oncogenic RNA viruses have shown no evidence of this enzyme activity. Two apparent exceptions were found. The first, visna virus, produces a chronic, progressive, neurological disease of sheep but has, heretofore, not been associated with tumors in sheep. The second major exceptions are the group of "foamy" viruses. These RNA-containing viruses are frequently found in healthy as well as diseased monkeys, cattle and cats, and they have not yet been associated with any disease. Visna and "foamy" viruses, then, are apparent exceptions to the rule that only tumor viruses contain reverse transcriptase. Whether visna and the related viruses of sheep and the various "foamy" viruses are potentially oncogenic in their natural hosts remains to be resolved.

The polymerase, as an antigen, like the gs antigen has both species specific and interspecies characteristics. Tumor-bearing animals can make antibody to the viral polymerase and some sera appear to be more broadly reactive than others. The murine polymerase has been partially purified and used to produce an antibody in rabbits. The antibody, an IgG immunoglobulin, is directed against the enzyme and not against the template. The antibody to the mouse leukemia virus polymerase will also

inhibit the enzymatic activity of hamster, rat and cat leukemia virus polymerase. Thus, the polymerases from different mammalian tumor viruses are antigenetically related. However, the crosses with other mammalian C-type viruses are only partial crosses allowing precise identification of the species of origin of an unknown C-type virus. The mouse mammary tumor virus, visna, and the avian leukemia virus polymerases are not inhibited at all by this serum. The antibody to the avian virus polymerase inhibits all the major avian C-type viruses, but not any mammalian C-type virus polymerase.

Two new C-type viruses of primates have recently been described by Kawakami and co-workers. One is from a woolly monkey fibrosarcoma; the other is from a gibbon ape lymphosarcoma. Both have a polymerase with the characteristic properties of tumor viruses and can be classified as C-type based on morphology. They also have the biological properties of C-type viruses and also have a cross-reacting gs antigen. The polymerase antibody studies, however, show a very weak or absent cross reaction with antibody to rodent or cat virus polymerase. Both the murine and feline C-type viruses can grow in primate cells without losing the immunologic specificity of their polymerase. These findings provide additional evidence that the polymerase coded for, at least in part, by the viral genome. From the experiments on polymerase inhibition, the woolly monkey and gibbon ape viruses appear not to be contaminating rodent or feline C-type viruses and appear to have a polymerase that is distinguishable from that of the C-type viruses of lower mammals. An antiserum prepared to the gibbon C-type polymerase inhibits gibbon and woolly virus enzyme well, but only poorly crosses with the rodent and feline previously described C-type virus polymerase.

The isolation of C-type viruses from both old world and new world monkeys from naturally occurring tumors greatly strengthens the possibility that related viruses will be directly isolated from human tumors. Several reports of C-type viruses in human cells have been presented in the past year. While some of these are more reasonable candidates than others, no proven "human" C-type is yet available. Until such viruses are found, the C-type viruses of primate origin should provide the best probes for the detection of C-type information in human cells.

In 1969, it was proposed that the cells of most or all vertebrate species contain C-type RNA virus genomes that are vertically transmitted from parent to offspring. Depending on the host genotype and various modifying environmental factors, either virus production or tumor formation, or both, may develop at some time during the life of these animals or in their cells when grown in culture. The evidence for this concept was derived both from cell culture experiments and from a variety of seroepidemiologic studies and was presented as a unifying concept that would be consistent with the facts as they were known at the time.

In the two years that have followed, a great deal more evidence has accumulated that provides strong support for the general theory. One

particular prediction that was made was that the genetic information for making an RNA tumor virus, being present in a repressed form in all cells, would be potentially inducible by carcinogenic and/or mutagenic agents. Recent evidence from single cell clones of mouse embryo cells of both the high susceptible strain, AKR, and the low susceptible strain, Balb/c, indicate that every cell clone in culture does contain the information for producing a C-type virus. Infectious virus can be induced from clonal lines of mouse, rat, and Chinese hamster cells, normal as well as transformed, which provides dramatic support for the original hypothesis.

The oncogene hypothesis makes several testable predictions. The first is that all somatic cells should contain the genetic information to produce a C-type virus that can be detected by using the DNA product made from C-type viral RNA of that particular species. For example, normal cat cells should have in their DNA a complete copy of cat leukemia C-type virus RNA, and the hamster should have in its DNA the genetic information for making a hamster C-type virus. A second and, perhaps, stronger prediction is that transformed cells, whether transformed by exogenously added tumor viruses or by radiation, chemical carcinogens or even "spontaneously", should contain new messenger RNA sequences that are not found in normal cells and that are common to all transformed and tumor cells of a particular species. This information should also be contained in the C-type tumor virus of that species. The new messenger RNA would be the product of the oncogene and in turn code for the production of the transforming protein(s). A third prediction is that it should be possible to isolate cell mutants that, at the nonpermissive temperature, because of a temperature sensitive repressor, would be superinducible or would possibly spontaneously produce C-type virus without exogenous inducer. A final prediction, if the general hypothesis is correct, and C-type viral information has been a stable part of the evolution of vertebrates, would be that the C-type viruses derived from closely related species would be closely related to one another in the antigenic properties of their characteristic proteins. Two such proteins are now available--the major group specific antigen and the reverse transcriptase. The isolation of C-type viruses from reptiles, birds, as well as mammals would suggest that they have evolved as the organism has evolved for many millions of years and that the species specific proteins will have evolved in much the same way that serum albumins, globulins, and other proteins have evolved. The genetic relatedness of the group specific antigens and the reverse transcriptases may well, then, be used as an index of the genetic relatedness of the species from which the C-type virus was derived. Obviously, those viruses derived from higher mammals, and especially primates, will be the most related to the viruses that come to be obtained from man. The VLLB is concentrating on the primate C-type viruses, because they should have enough genetic relatedness so that an antiserum produced to the purified polymerase or the purified group specific antigen should show some ability to recognize C-type viruses isolated from human tissues.

Other Research Developments in the Branch

Characterization of continuous, contact-inhibited mouse cell lines from Balb/c and NIH/Swiss embryo cells has provided excellent model systems for study of the effects of tumorigenic viruses both in vitro and in vivo. These cell lines, and a wide variety of well-characterized subclones from them, are supplied to numerous investigators throughout the world, and have become standard cell lines for biochemical and biological investigations of cellular growth control mechanisms.

New types of MSV-transformed cells, the nonproducer cells, have been discovered. These cells are morphologically transformed and are highly tumorigenic, yet they lack all the known antigens of the murine sarcoma-leukemia complex. The sarcoma genome can be readily rescued by the addition of "helper" leukemia virus. These nonproducer cells provide a very good model for the study of naturally occurring cancers. Viral specific information can be detected by nucleic acid hybridization at less than 1% of the level in the transformed virus producing cells. Optimal techniques for detection of viral information in these cells are being applied to studies on the detection of viral information in human tumors and human tissues.

Studies on the S+L- cells developed by Bassin, et al have revealed that the particles they produce have only the main group specific (gs) and the interspecies antigen. while the other gs antigens seem to be missing or radically different. Type specificity of the S+L- virus is also not related to previously described MuLV groups. The S+L- particle itself contains a neatly reduced amount of reverse transcriptase; the S+L- cells are also deficient in this enzyme. A significant lack of homology between MuLV and the standard coated MSV was seen when mixtures of MSV-MuLV (4:1) were examined by reciprocal cross-hybridization with the respective DNA reverse transcriptase products. Additionally, atypical large molecular weight nucleic acids may be a natural component of MSV as well as previously observed ribosomal and transfer RNA species.

A major advance has been the development of virus stocks which have a significant (4- to 50-fold) excess of the transforming MSV over its associated murine leukemia "helper" virus (MuLV). With a favorable MSV excess the isolation of cells transformed only with MSV and the study of MSV genome itself are greatly facilitated. The result has been the development of numerous cell lines that are "transformed" by MSV but have only a minimal expression of the tumor virus information. The virus from these cultures with an excess of sarcoma virus should provide a valuable probe for the search for sarcoma virus specific information in apparently "virus-free" tumors.

Radiation leukemia virus, a "natural" virus induced from C57Bl mice has been found to be able to replicate, albeit with great difficulty, in 3T3 cells. Because the radiation leukemia virus is able to grow much better in S+L- transformed 3T3 cells, it may be that the great majority

of naturally induced C-type viruses existed as MSV complementation requiring variants. The isolation of new natural viruses may require the above model techniques. An obvious extension that is actually being investigated is the isolation of human cells infected only with MSV and the attempted induction from them of the postulated endogenous human helper C-type virus. "Revertants" of MSV transformed that have lost partial or complete virus extension have been obtained from a number of systems. These cells also have lost tumorigenicity. In some cases the input oncogenic information appears to be lost; in others it is suppressed. The latter situation provides an excellent opportunity for the study of the mechanism by which tumor virus information is repressed.

A new strain of murine sarcoma virus isolated from a B/W mouse is accompanied by a non-cytopathogenic "helper" virus required for release of infectious virus. Unlike other strains of sarcoma virus, the new MSV retains the ability to express the murine gs-1 antigen in hamster and cat cells. The presence of a convenient antigenic marker is useful in studies involving trans-species rescue of the MSV genome using putative "human" C-type viruses. Marked mouse strain differences are noted in the response to this MSV strain. In Balb/c and C3H mice tumor regression, virus titers, viral neutralizing and cytotoxic antibody titers are correlated. But in B/W mice, the tumors persist despite very high titers of neutralizing and cytotoxic antibodies, and absence of detectable infectious virus.

The combined use of immunology and electron microscopy has greatly increased the sensitivity and specificity with which virus specific and virus directed antigens may be localized. In addition to ferritin and southern bean mosaic virus coupled to hybrid antibody, tobacco mosaic virus (TMV) has been utilized and established as a third marker for immuno-electron microscopy. Fragmented TMV (50 mμ) can be used with the other two markers for conventional electron microscopy, since these three markers are clearly distinguishable from each other by their shapes and specificities. Thus, the use of these three different markers opened a way to examine the relationship among different antigenic sites on the surface of the virus and the virus-containing cells. During virus budding, it is seen that the C-type virus incorporates cell surface components into the viral envelope.

Micro techniques for the isolation of temperature sensitive leukemia virus mutants have been developed. By these methods, it has been possible so far to isolate 27 temperature-sensitive leukemia virus mutants from clonal stocks of Kirsten and Rauscher leukemia viruses. Each mutant transmits to new cells with efficiency comparable to that of wild-type MuLV at the permissive temperature, but is at least 4-5 logs less efficient than wild-type at forming XC plaques at the nonpermissive temperature. The mutants all have very low rates of reversion to wild-type. Temperature sensitive sarcoma virus mutants have also been isolated. With these it is hoped that the viral protein directly responsible for the maintainence of the transformed state can be studied.

A continuous cell line, HBT-3, was established in culture from a human breast adenocarcinoma. The cells are epithelioid in appearance, grow rapidly, and form multilayered colonies. HBT-3 cells have a cloning efficiency of approximately 70% and an abnormal karyotype. Preliminary experiments with this cell line suggest the possible presence of 60-70S RNA and an enzyme that is similar to the known viral reverse transcriptases.

By nucleic acid hybridization, it has been possible to detect C-type viral specific RNA from every mouse cell line tested to date, including such lines as Balb/3T3 and NIH/Swiss mouse embryo fibroblasts (NIH/3T3). Cell lines transformed by SV40 and Kirsten or Moloney sarcoma virus show increased levels of hybridization. The finding of partial expression of murine C-type information in "normal" mouse cells not treated with inducing agents suggests possible post-transcriptional control of the expression of the endogenous virus information.

Rauscher-MuLV reverse transcriptase is denatured by, and renatures upon removal of, guanidine hydrochloride (GuHCl). The enzyme is composed of one polypeptide chain of molecular weight approximately 70,000 - 80,000. The renatured enzyme obtained from an agarose column run in 6M GuHCl and reducing agent still possesses RNA and DNA directed DNA polymerase activities associated with the native enzyme. The renatured enzyme reacts with antibodies specific for the native enzyme. Studies are in progress to irreversibly denature host cell polymerases but not the viral enzyme. If successful, this will provide a rapid, specific assay for reverse transcriptase in host cells.

Immunoadsorbant columns using anti-viral polymerase antibody coupled to Sepharose have been used to purify reverse transcriptase from cells and tissues. Large scale screening of human cell extracts using affinity chromatography will be undertaken when a high titered antibody directed against the primate C-type viral reverse transcriptase is available.

Continuous therapy with interferon inducers effectively suppresses several virus-induced and transplantable tumors. However, pretreatment with single doses of interferon inducers often results in marked enhancement of oncogenesis by RNA tumor viruses. Pretreatment with inducers can result in the more rapid growth of several transplantable tumors in certain systems. Several synthetic deoxynucleotides induce low levels of circulating interferon and prevent death in mice from arborvirus infections. They also enhance tumor induction by murine sarcoma virus, an effect produced by all the interferon inducers tested to date. These studies may help elucidate the mechanism of interferon induction in the animal.

AKR mice have been considered immunologically tolerant to MuLV since they are G antigen positive during their entire life span. It has become evident, however, that the kidney-eluate of untreated AKR mice contains at least two different antibodies to intraviral G antigen(s) and the Gross cell surface antigen, although no free antibodies are detected in the body fluid. These findings indicate that there may not be absolute

immunological tolerance. Immunologic studies on the behavior of malignant cells might suggest methods of treating tumor patients. The apparent absence of absolute immunological tolerance to C-type virus-associated antigens raises the question of whether it may be possible to break relative immunological tolerance to both viruses and malignant cells. In combination with studies on activation of viral genome producing antigens this may open a way to the development of immunologic methods of prophylaxis and treatment.

Controlled studies continued to demonstrate that patients with Hodgkin's disease have higher EBV antibodies than matched controls. Studies with cell-mediated immunity to a variety of antigens indicated that anergy was associated with a poor prognosis, but there was no correlation between EBV titers and general cell-mediated immunity. At least three Rhesus monkeys have been successfully infected with EBV. This was demonstrated by persistence of elevated antibody levels to the virus while monkeys given non-infectious EBV did not maintain high titers. The ability to infect Rhesus monkeys with EBV is of significance because it raises the possibility of a good model system for human studies. Rhesus monkeys have natural antibody to a virus indistinguishable from EBV at the present time, and by manipulation of the animals with inbreeding and immunological alteration, it may be possible to use monkeys as a model for the study of EBV-induced diseases in humans.

Ten sets of identical twins, one member of each pair having leukemia, were studied by at least two different immunological tests. Similar studies were performed on six sets of normal identical twins. In six of the leukemia families studied, lymphocytes from family members and unrelated donors were cytotoxic for cells from leukemic twin and not for cells from the normal twin. Delayed hypersensitivity reactions were elicited by extracts of patients' leukemic cells but not by extracts of remission cells and from the normal twin cells. Neither cytotoxicity nor delayed hypersensitivity were found in any of the normal identical twins.

Other Activities of the Branch

During this reporting period, senior investigators of the Branch published a total of 85 papers which covered various aspects of viral oncology.

Members of the Branch presented by invitation over 50 lectures to research groups in this country and abroad, and over 30 abstracts were presented at various scientific meetings. The Branch entertained approximately 150 visitors for discussions in various aspects of viral oncology; this included representatives from every major research institution in this country and from 14 foreign countries. The Branch also provided training for periods up to six months in a variety of experimental procedures to outside visitors. This training consisted of orientation in immunological, biological and biochemical procedures as well as virus handling techniques.

Many of the investigations described in the Viral Oncology portion of this report depend on the availability of clinical and laboratory materials of optimal purity, viability, potency, etc. The interaction which results in neoplasia needs several participants: the virus(es), the cell, or their already reacted product, the transformed cell. An understanding of each of the components requires highly specialized, well defined and custom made reagents with recognizable markers to serve as specific probes. Indeed, comparative studies in an integrated program of international scope, as encompassed in the SVCP, can make more meaningful and rapid progress when adequate quantities of standardized reagents, cell cultures and test animals are available.

The Resources and Logistics Activity provided viruses and antisera, human tissues, special laboratory animals (including infectious leukosis virus-free eggs), all produced, characterized, stored and distributed under various contract operations. Diagnostic services for the detection of murine, non-human primate, and cat viruses, and the viral reagents for these tests, were also provided.

In addition, several of the senior investigators within the Branch spent a portion of their time in support of the Special Virus Cancer Program. The members serve as Chairmen, Vice Chairmen, Work Group members and Secretaries of the major segments of the Program. They provide scientific guidance as Project and Assistant Project Officers on research contracts supported by the Special Virus Cancer Program.

The activities of the SVCP and the direction of the internal research program of the Branch are aimed at the common goal of the determination of the viral etiology of human cancer. It is apparent that the efforts of the Branch members have played a significant role in the progress of the SVCP to date. The broad scientific perspective developed by these investigators in their SVCP activities has also contributed significantly to the direction of the Branch program for the attainment of research goals.

The effective functioning of senior personnel in dual capacities, i.e., in-house research and program administration requires a delicate balance of effort. It must be realized through constant monitoring, that such a balance does exist and over-emphasis in either direction would be to the detriment of both programs. It has become clearer during the past year that an understanding of the suspected relationship between tumor viruses and human neoplasia requires an interaction between, among others, highly skilled molecular biologists, epidemiologists, cell biologists and physicians, along with sound and constructive administrative support, the answers will come from no one discipline alone.

Serial No. NCI-4821
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Genetic and Environmental Factors in Susceptibility to Endogenous and Exogenous Tumor Virus Information.

Previous Serial Number: 4904, 4906, 4919,

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Bionetics Laboratories, Bethesda, Maryland
Naval Biological Laboratory, Oakland, California
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Man Years:

Total:	1.2
Professional:	1.0
Other:	0.2

Project Description

Objectives:

1. To develop a rapid assay for cellular sensitivity to viral transformation suitable for screening human populations.

2. Determination of the reason for the difference between normal and susceptible human cell strains with regard to transformation by oncogenic virus.

3. To determine the basis for the difference in susceptibility of cells to exogenously added oncogenic RNA viruses.

4. To utilize susceptible human cells and the most optimal conditions to search for other transforming viruses for human cells.

5. To study the ability of RNA and DNA from tumor viruses to transform human cells.

6. To study how agents such as radiation and chemical carcinogens act to facilitate transformation.

7. To determine the mechanism by which cells can prevent the expression of endogenous oncogenic information.

Methods Employed:

Standard techniques of tissue culture, biochemistry, and immunology, virus isolation and virus antigen detection are being used.

Major Findings:

1. A markedly increased susceptibility to transformation has been found with cells from eight individuals with Fanconi's anemia and five individuals with Down's syndrome. Both of these genetic diseases are associated with a high incidence of spontaneous tumors. Cells from individuals with immunologic deficiencies and a high risk of cancer, such as the various forms of agammaglobulinemia, show normal transformation susceptibility.

2. Susceptible cells by the SV40 transformation test also appear to be more susceptible to transformation by murine sarcoma viruses (Kirsten and Moloney strains). Over 700 human fibroblast strains have been tested for their SV40 transformation susceptibility. These include cells from normal persons of all ages, cells from certain partially inbred human populations, cells from relatives of tumor patients and cells from various "high risk" clinical conditions.

3. Methods to detect viral low levels of C-type virus expression in normal as well as transformed cells have been developed.

Significance to Biomedical Research and the Program of the Institute:

1. These studies suggest a possible simple test for "cancer proneness" in man. They may allow us to recognize a "high risk" group for cancer in the human population.

2. The most susceptible cells would appear to offer the best possibility for use as test cells in the search for hitherto undetected tumor viruses of man.

3. Methods for the sensitive and accurate detection of virus specific information are essential to resolve the etiologic role of viruses in human cancer.

Proposed Course:

1. To develop a simple in vitro test to measure a person's "risk" of developing cancer. The testing of additional populations will make clear how far the initial observations can be extended and whether this approach will be clinically useful. The factor(s) in cells that are responsible both for susceptibility and resistance will be studied with the goal of characterizing these factors in biochemical terms.

2. To use a combination of methods that have become available recently to resolve whether C-type viruses are present in human tumors. If they are, to then determine how they are transmitted and whether they are important etiologic agents in human cancer. The approach to the prevention and/or cure of C-type virus induced human tumors requires an answer to the first two questions.

3. To determine the mechanism by which oncogenic virus information is able to transfer normal cells into tumor cells.

Honors and Awards:

DHEW Superior Service Award, 1971
Gustav Stern Award for Virology, 1972.

Publications:

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Todaro, G.J.: Immunological identification of the species or origin of leukemia viruses. Scienze & Technica 72. In press.

Serial No. NCI-4822

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: (A) EBV Studies in Humans and Non-Primates
(B) The Role of RNA Viruses in Human Leukemia and Breast Cancer

Previous Serial Number: NCI-4811

Principal Investigator: Dr. Paul H. Levine

Other Investigators: None

Cooperating Units: Inside NIH
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Dr. Paul Gerber, DBS
Dr. Gary Pearson, VBB, NCI
Dr. Dharam Ablashi, VLLB, NCI
Dr. Costan W. Berard, LP, NCI
Dr. Gregory T. O'Conor, LP, NCI
Members of the Medicine, Viral Biology, Immunology-GL&C, and Epidemiology Branches, NCI

Outside NIH
University of Colorado Medical Center, Denver, Colorado
Dr. E. Perlin, National Naval Medical Center, Bethesda, Maryland
Dr. L. Dabich, Simpson Memorial Inst., Ann Arbor, Mich.
Dr. Peter Ebbesen, University of Denmark, Copenhagen
Dr. Paul Terasaki, UCLA, Los Angeles, Calif.
Bionetics Research Laboratories, Bethesda, Maryland
Pfizer and Company, Maywood, New Jersey

Man Years:

Total:	1
Professional:	1
Other:	0

Serial No. NCI-4822

Project Description

Objectives:

Project A

1. To develop seroepidemiologic studies using viral capsid antibody and antibody to the early antigen to determine whether EBV is etiologically related to human lymphoma.

2. To investigate the role of cellular immunity to EBV as a possible controlling factor of antibodies to the viral capsid antibody.

3. To correlate the immune response to EBV-associated antigens and lymphoma-associated antigens in order to determine if there is a relationship between the two.

4. To evaluate the genetic factors controlling the immune response to EBV and susceptibility to lymphoma.

Project B

1. To pursue preliminary studies indicating that there is an antigen in human tumor cells which cross-reacts with murine and/or feline leukemia viruses.

2. Using identical twins, to identify a leukemia specific antigen with humoral and cell-mediated immunological assays. To determine whether this antigen can be detected by family members and controls. To evaluate techniques in this system which might be more sensitive and practical in providing evidence as to the etiology and control of human leukemia.

Methods Employed:

Project A

1. A registry of American Burkitt Lymphoma patients was established in order to obtain a population to compare clinically, epidemiologically, and virologically with the African Burkitt patients. Blood samples were collected from a variety of lymphoma patients, particularly those with American Burkitt's Lymphoma and Hodgkin's disease, as well as normal controls. Cell lines known to contain EB virus, fresh tumor biopsies, and cell lines without evidence of EBV were used as a source of antigen in these studies. Sera were tested for antibody to the viral capsid antigen and the early antigen by immunofluorescence. Lymphocyte cytotoxicity, skin testing, and lymphocyte stimulation were employed as tests of cellular immunity.

2. Rhesus monkeys were inoculated with large doses of infective HRlK virus. Serum samples were collected for antibody determination and careful monitoring was performed to look for clinical and laboratory evidence of disease. Heavy doses of chemotherapy were administered to some of the monkeys to determine whether the response to virus could be altered by drugs, either immunologically or clinically.

Project B

1. Serum samples were obtained from patients with a variety of diseases, families with multiple cases of cancer, and normal individuals who were probably exposed to animal oncogenic tumor viruses (cat breeders, viral oncology laboratory workers, etc.). Sera were distributed to a number of NCI and SVCP investigators for application in the immunological studies. Among the tests performed were passive hemagglutination, immunodiffusion, neutralization, isotopic studies (PRILAT), complement fixation, and complement fixation inhibition. Target cell lines included the Belev (F230) cell line derived from a breast cancer patient which had previously been identified as containing B or C type particles by electron microscopy, cell lines infected with Rauscher leukemia virus and cell lines infected with feline leukemia virus.

2. Ten patients with acute leukemia and their identical twins were evaluated by a variety of techniques. Lymphocyte cytotoxicity, mixed lymphocyte culture and skin testing were used to determine whether cellular immunity tests could identify a tumor specific antigen, and whether there was evidence that this antigen caused immune reactivity in family members and controls. Cells from the patient and his identical twin were used as sources of antigens directly and materials were also placed in tissue culture. Skin fibroblasts from family members were tested to determine whether any genetic factors could be associated with acute leukemia. Electron microscopy and immunofluorescence, using antisera against Rauscher and feline leukemia virus, were utilized to determine whether any of the antigens detected by cellular immunity tests were related to any known animal leukemia viruses.

Major Findings:

1. Studies in 29 American patients with lymphomas histologically indistinguishable from African Burkitt's Lymphoma indicated that American and African Burkitt's Lymphoma share clinical and virological features. Antibody titers were higher in the American Burkitt's patients than age matched normal patients or patients with acute lymphocytic leukemia, although they were not as high as those in the African Burkitt patients. All cases with typical histological features and adequate pathological materials (including imprints) were positive for antibody to EBV. The American Burkitt patients (less than 8 years old) have particularly high titers with a geometric titer of 1:425 compared to 1:11 and 1:4 in the two age matched American control groups ($p < .005$). Although the number of

American Burkitt patients is too small to make reliable conclusions possible, the data to date supports the hypothesis that at least the young American patients with Burkitt's Lymphoma have the same disease as African patients with Burkitt's Lymphoma, and that EBV bears the same relationship to American Burkitt's as it does to African Burkitt's.

2. Controlled studies continued to demonstrate that patients with Hodgkin's disease had higher EBV antibodies than matched controls. Studies with cell-mediated immunity to a variety of antigens indicated that anergy was associated with a poor prognosis, but there was no correlation between EBV titers and general cell-mediated immunity. In preliminary studies, where cell-mediated immunity to specific EBV-associated antigens and humoral antibody to EBV were compared in the same patient, however, there was a generally inverse correlation between cell-mediated immunity to EBV and humoral antibody to EBV.

3. A tumor histologically indistinguishable from African Burkitt's Lymphoma was identified as occurring simultaneously in a brother and sister in California and in two neighbors in Winchester, Virginia. Immunological studies showed that three of the four patients had no antibody to EBV.

4. Controlled studies indicate that at least three Rhesus monkeys were successfully infected with EBV. This was demonstrated by persistence of elevated antibody levels to the virus while monkeys given non-infective EBV did not maintain high titers.

5. Ten sets of identical twins, one member of each pair having leukemia, were studied by at least two different immunological tests. Similar studies were performed on six sets of normal identical twins. In six of the leukemia families studied, lymphocytes from family members and unrelated donors were cytotoxic for cells from leukemic twin and not for cells from the normal twin. Delayed hypersensitivity reactions were elicited by extracts of patients' leukemic cells but not by extracts of remission cells from the normal twin cells. None of these findings were present in the normal identical twins.

6. Although most of the tests utilized were unable to detect antibodies to feline leukemia virus, one test, the paired radio isotope labelling antibody technique, indicated that some human sera did react with the feline leukemia virus, although specificity could not be confirmed at the present time.

Significance to Biomedical Research and the Program of the Institute

1. The studies in American Burkitts are important since a closer scrutiny of the same disease in geographically distinct areas allows a more careful look at the etiology by separating various factors. If American Burkitt's has the same etiology as African Burkitt's, it can be evaluated without

concern for the role of malaria as a causative factor.

2. Studies in EBV have now linked the virus to a number of diseases, including nasopharyngeal carcinoma, Burkitt's Lymphoma, Hodgkin's disease, chronic lymphocytic leukemia, and sarcoidosis. The evidence for etiology, however, is not clear. The inverse correlation of cell-mediated immunity and humoral antibodies indicates that high EBV antibody levels may be the result of poor cell-mediated immunity to the virus. Since development of tumors is generally related to cell-mediated immunity rather than humoral antibody, the possible mechanisms for EBV oncogenesis may be elucidated. It is also possible now to apply the findings in Hodgkin's disease to other diseases with high EBV titers to determine whether the same inverse relationship between cell-mediated immunity and humoral antibody are present.

3. Clustering of American Burkitt's Lymphoma is important because it indicates that environmental factors may be involved in the etiology of Burkitt's Lymphoma in the United States. The absence of EBV antibody in the patients could either reflect the rapid progression of disease or it may indicate that a virus other than EBV may be involved in the etiology of Burkitt's Lymphoma.

4. The ability to infect Rhesus monkeys with EBV is of significance because it raises the possibility of a good model system for human studies. Rhesus monkeys have natural antibody to a virus indistinguishable from EBV at the present time, and by manipulation of the animals with inbreeding and immunological alteration, it may be possible to use monkeys as model for EBV-induced diseases in humans.

5. The twin studies indicate that humans may produce an immune response to leukemia associated antigens. At least one of the antigens detected by this system may be environmental. These studies in twins permit a clear analysis of the specificity of the antigens being detected and are important to studies on the etiology and cure of human leukemia.

6. The ability to detect antibodies to animal leukemia viruses in humans could allow a clear evaluation of the role of animal tumor viruses in human disease.

Proposed Course:

Project A

1. The American Burkitt Registry will be expanded with more intensive evaluation of the epidemiological, virological, and genetic aspects of American Burkitt's Lymphoma.

2. Careful evaluation of a larger number of Hodgkin's disease patients for immunity to tumor and virus associated antigens will be pursued. Cooperative studies will be initiated to isolate potential oncogenic viruses

from the materials collected so that immunological tests can be related to studies of candidate oncogenic viruses.

3. The studies in Rhesus monkeys will be pursued in collaboration with Dr. Gary Pearson, Dr. Dharam Ablashi and investigators at Bionetics Research Laboratories. Similar immunological systems will be established in humans and monkeys so that if EBV is etiologically related in human lymphomas, the monkeys can be manipulated to permit the oncogenicity of EBV to be more readily demonstrated. Vaccine trials to demonstrate potency of EBV-related vaccines could be developed in these monkey systems.

Project B

1. The study of identical twins will continue in an attempt to define the specificity of the leukemia associated antigens and the immune response of humans to these antigens. New tests will be applied to the study of twins as they are developed, since these families are ideal for the study of genetic and immunological factors associated with etiology of acute leukemia. In addition, twins with other forms of cancer will be sought and methodology successfully applied to the twin leukemia study will be applied to the other twin families as well.

2. Additional sera will be collected from people at high risk of infection with animal tumor viruses. These sera will be stored and as methods for detecting specific antibody to these viruses are improved the sera will be used in studies with immunological tests.

Honors and Awards:

Dr. Levine presented a review entitled "The Current Status of Burkitt's Lymphoma" at Georgetown University in Washington, D.C., February 22, 1971.

Dr. Levine presented a talk entitled "EBV and Burkitt's Lymphoma" at M.D. Anderson Hospital and Tumor Institute in Houston, Texas, on January 24, 1972.

Dr. Levine presented a talk entitled "The Use of Animal Models for Epidemiological Studies in Human Leukemia and Lymphoma" at the Annual Joint Working Conference of the Special Virus Cancer Program in Hershey, Pennsylvania, on October 24, 1971.

Dr. Levine presented a manuscript entitled "Studies on the Relationship of EBV Titers to Cell-Mediated Immunity in Patients with Hodgkin's Disease" at the Hodgkin's Disease Symposium at Stanford University in Palo Alto, California, on March 20, 1972.

Publications:

Chan, S.P., Maca, R.D., Levine, P.H., Ting, R.C.: Immunologic studies of human breast cancer. I. Serum reactivity against a lymphoid cell line (Belev) derived from a breast cancer patient as detected by complement-fixation test. J. Nat. Cancer Inst. 47: 511-517, 1971.

Levine, P.H.: The relationship of Epstein-Barr (EBV) antibodies to disease state in Hodgkin's Disease, chronic lymphocytic leukemia and American Burkitt's Lymphoma. Proc. of the Cambridge Symposium on Oncogenesis and Herpes-Type Viruses, England. 1971. In press.

Stevens, D.A., O'Conor, G.T., Levine, P.H., and Rosen, R.B.: Acute leukemia with "Burkitt's Lymphoma" cells: (a new entity) and Burkitt's Lymphoma - simultaneous onset in American siblings. Ann. Intern. Med., 1972. In press.

Levine, P.H., Merrill, D.A., Bethlenfalvay, N.C., Dabich, L., Stevens, D.A., and Waggoner, D.E.: A longitudinal comparison of antibodies to Epstein-Barr Virus and clinical parameters in chronic lymphocytic leukemia and chronic myelocytic leukemia. Blood, 38: 479-484, 1971.

Stevens, D.A., Easton, J.M., Levine, P.H., Waggoner, D.E., Manaker, R.A., and Schidlovsky, G.: Antilymphocyte serum and lymphoid cell-virus carrier culture: cell kinetics, morphology, EB virus replication, interferon. Cell. Immun., 1972. In press.

Rosenberg, E., Herberman, R.B., Levine, P.H., Wunderlich, J., McCoy, J.: Lymphocyte cytotoxicity reaction to leukemia-associated antigens in identical twins. Int. J. Can., 1972. In press.

Serial No. NCI-4824

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Etiology
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies with RNA oncogenic viruses

Previous Serial Number: Same

Principal Investigator: Dr. Adi F. Gazdar

Other Investigators: Dr. Yoji Ikawa
Dr. Paul Peebles
Dr. Richard Buswell

Cooperating Units:

Inside NIH
Dr. Padman Sarma, VCB, NCI
Dr. Alfred Steinberg, LAR, NIAMD
Dr. Sam Baron, LBV, NIAID
Dr. Harish Chopra, VB, NCI
Dr. Ronald Herberman, LCBGY, NCI
Dr. Robert Herman, LPD, NIAID

Outside NIH
Dr. Gerald Spahn, Microbiological Associates,
Walkersville, Maryland

Man Years:

Total:	3.0
Professional:	1.0
Other:	2.0

Project Description

Objectives:

1. To characterize the properties of "defective" murine sarcoma viruses.

2. To investigate the relationship between certain viruses, interferon inducers, autoimmune disease and neoplasia in New Zealand mice.

3. To investigate the relationship between the interferon system, oncogenic viruses, and susceptibility to oncogenesis.

4. To investigate the effects of cyclic AMP on virus transformed cells.

Methods Employed:

1. Immunologic tests include complement fixation and fluorescent antibody tests.

2. The progress of autoimmune disease is followed by radio-immunoassay tests for antibodies to RNA and DNA, as well as by light and electron microscopy.

3. Interferon assays are performed using an inhibition of hemagglutinin yield method. The effects of interferon inducers on the interferon system, immune system, tumor development, and virus yield are studied.

4. Sugar transport in malignant cells is measured by uptake of labelled sugars. Tumor antigens are measured by a cytotoxic test.

Major Findings:

1. A recently discovered strain of murine sarcoma virus induces tumors in hamsters. The hamster tumors and their cell cultures release a "non-infectious" sarcoma virus having the gs-1 antigen of the murine leukemia-sarcoma group. After several in vivo passages, one tumor line was found to contain the gs-1 antigen of both hamster and mouse C-type viruses. Cat cells transformed by feline leukemia pseudotype MSV contain both feline and murine gs antigens. The genome of the new MSV strain can express gs antigen in heterologous hosts and in the presence of heterologous C type viruses. This property is not demonstrated by the other known MSV strains and provides a convenient antigenic marker of the sarcoma genome in heterologous cells. The sarcoma virus contains a relative deficiency of reverse transcriptase, and this may explain its lack of infectivity in the absence of a "helper" virus.

2. A strain of MSV isolated from a NZW/NZB (B/W) F_1 hybrid mouse induces a peculiar splenic lesion in B/W mice, characterized by massive splenomegaly fibrosis and inflammation. Unlike other mice, in B.W. mice infected with large doses of the virus the tumors fail to regress despite high titers of neutralizing and cytotoxic antibodies. After 6 weeks, progressive splenomegaly develops. Infectious virus cannot be recovered 3-4 weeks post infection, but death invariably follows. Lesser, non-fatal doses of virus enhance the onset of anti-RNA antibodies. Several interferon inducers, notably rI·rC, rA·rU and COAM, also stimulate the formation of antibodies to RNA. Other inducers such as NDV, tilorone and statalon do not and in fact may inhibit the onset of anti-RNA antibodies.

3. Pretreatment with several interferon inducers enhance viral oncogenesis. The mechanism is not understood, but enhancement can also be demonstrated in vitro. Polydeoxyribonucleotides, not generally recognized as interferon inducers, also enhance the effects of MSV in vivo and in vitro. Our recent studies indicate that polydeoxyribonucleotides induce low levels of interferon and prevent mortality from Semliki Forest virus infection. Interferon inducers also greatly enhance the growth of certain tumor transplant lines.

4. Dibutyryl cyclic AMP (cAMP) has been reported to reverse the malignant features of transformed cell cultures. Investigations using a MSV transformed "non-producer" cell line confirm the morphological change towards the appearance of the parent non-transformed cell line after cAMP treatment. Slower growth rate and greater adhesiveness also follow cAMP treatment. MuLV replication and MSV "rescue" are decreased in the cAMP treated cells. However, cAMP treatment does not reduce the elevated glucose transport in the transformed cells, and viral antigens can be detected after several days of treatment. The data are consistent with a partial but not complete reversal of transformation following cAMP treatment.

Proposed Course:

1. The physical and antigenic properties of the sarcoma virus synthesized in the presence and absence of associated "helper virus" will be compared and contrasted.

2. The effect of several interferon inducers and RNA oncogenic viruses on antinuclear antibodies, mortality, and renal disease in B/W mice will be studied.

3. The mechanism of enhancement of viral oncogenesis and tumor cell growth by interferon inducers will be studied.

4. Further studies beyond the scope of those already outlined are not planned.

Significance to Biomedical Research and the Program of the Institute:

Investigations on the nature and mode of action of certain animal type C oncogenic viruses, especially as they affect species other than that of origin will contribute to a more complete understanding of certain human cancers. Indeed, prevention or control of cancer is dependent upon the elucidation of mechanisms by which viruses manipulate species barriers and further definition of host response to oncogenic agents. Characterizing the properties of "defective" oncogenic viruses will aid in the search for putative human sarcoma viruses.

Honors and Awards:

Dr. Gazdar presented a paper entitled "Enhancement of viral oncogenesis by interferon inducers" to the 3rd International Colloquim on Interferon and Interferon Inducers, Leuven, Belgium.

Dr. Gazdar presented a paper entitled "Properties of a defective sarcoma virus" to the American Association of Cancer Research.

Publications:

Gazdar, A.F., Phillips, L.A., Sarma, P.S., Peebles, P.T., and Chopra, H.C.: Presence of sarcoma genome in a "non-infectious" mammalian virus. Nature New Biology 234: 69-72, 1971.

Gazdar, A.F., Weinstein, A.J., Sims, H.L., and Steinberg, A.D.: Enhancement and suppression of murine sarcoma virus induced tumors by Poly I·poly C. Proc. Soc. Exp. Biol. Med. 139: 279-285, 1972.

Gazdar, A.F., Steinberg, A.D., Spahn, G.J., and Baron, S.: Interferon inducers: enhancement of viral oncogenesis in mice and rats. Proc. Soc. Exp. Biol. Med. 139: 1132-1137, 1972.

Gazdar, A.F., Chopra, H.C., Sarma, P.S.: Properties of a murine sarcoma virus isolated from a tumor arising in a NZW/NZB F_1 hybrid mouse. I. isolation and pathology of tumors in rodents. Int. J. Cancer 9: 219-233, 1972.

Gazdar, A.F., Sarma, P.S., and Bassin, R.H.: Properties of a murine sarcoma virus isolated from a tumor arising in a NZW/NZB F_1 hybrid mouse. II. Physical and biological characteristics. Int. J. Cancer 9: 234-241, 1972.

Talal, N., Steinberg, A.D., and Gazdar, A.F.: Specific immunosuppression, Poly I·poly C, and viruses in New Zealand mice. Fed. Proc. 30: 1842-1845, 1971.

Talal, N., Steinberg, A.D., Jacobs, M.E., Chused, T.M., and Gazdar, A.F.: Immune cell co-operation, viruses, and antibodies to nucleic acid in New Zealand mice. J. Exp. Med. 134: 52x-64s, 1971.

Levy, H.B., Adamson, R., Carbone, P., deVita, V., Gazdar, A., Rhim, J., Weinstein, A., and Riley, F.: Studies on the anti-tumor effect of Poly I·poly C. Beers, R.F. and Braun, W. (Eds.): Biological Effects of Polynucleotides. New York, Springer-Verlag, 1971, pp. 55-65.

Serial No. NCI-4824

Peebles, P.T., Haapala, D.K., and Gazdar, A.F.: Deficiency of viral RNA-dependent DNA Polymerase in non-infectious virus-like particles released from murine sarcoma virus transformed hamster cells. J. of Virol. In press

Serial No. NCI-4825
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Physicochemical studies of viral nucleic acids and enzymes

Previous Serial Number: Same

Principal Investigator: Dr. Daniel Haapala

Other Investigators: Dr. Peter J. Fischinger
Dr. Robert H. Bassin
Dr. Shigeko Nomura
Dr. Paul T. Peebles
Dr. Leo A. Phillips
Dr. Brenda Gerwin

Cooperating Units: Inside NIH
Staff, VLLB, NCI

Outside NIH
None

Man Years:

Total:	2.3
Professional:	1.0
Other:	1.3

Project Description

Objectives:

To characterize viral nucleic acids and enzymes for use as diagnostic tools and to gain insight into the mechanism of viral oncogenesis.

Methods Employed:

Standard biochemical and biophysical methods were used.

Major Findings:

Murine sarcoma virus:

1. Non-infectious MSV particles are deficient in reverse transcriptase.

2. Cells transformed by MSV are deficient in reverse transcriptase.

3. A nucleic acid species with unusual physical chemical properties is found in supernatants of MSV transformed cells.

4. Certain MSV cells lose their transformed properties but contain MSV in a rescuable form.

5. MSV genome carrying information for formation of budding particles and having $\geq$ 70% UV target size of MuLV nevertheless shares little or no genetic information with MuLV as measured by DNA-RNA hybridization.

Leukemia viruses:

1. Certain heteropolyanions which inhibit virus replication have been shown to inhibit virus reverse transcriptase.

2. The nucleotide sequences of different leukemia virus species are heterogeneous.

3. Leukemia viruses from different species share an adeninethymidine rich segment.

4. Extensive intra-strand base pairing exists within the 65S MuLV genome.

Significance to Biomedical Research and the Program of the Institute:

The physicochemical properties of viral nucleic acids and enzymes provide tools with which to detect, characterize and possibly inhibit neoplastic changes of viral etiology.

Proposed Course:

Studies of human tumor material will be emphasized based on the findings reported above.

Honors and Awards:

None

Publications:

Haapala, D.K. and Falkow, S.: Physicochemical studies on the resistance transfer factor RTF in Proteus mirabilis. J. Bacteriol. 106: 294-295, 1971.

Bassin, R.H., Phillips, L.A., Kramer, M.J., Haapala, D.K., Peebles, P.T., Nomura, S. and Fischinger, P.J.: Transformation of mouse 3T3 cells by murine sarcoma virus: Release of virus-like particles in the absence of replicating murine leukemia helper virus. Proc. Nat. Acad. Sci. U.S.A. 68: 1520-1524, 1971.

Peebles, P.T., Bassin, R.H., Haapala, D.K., Phillips, L.A., Nomura, S. and Fischinger, P.J.: Rescue of murine sarcoma virus from a sarcoma positive leukemia negative cell line: Requirement for replicating leukemia virus. J. Virol. 8: 690-694, 1971.

Fischinger, P.J. and Haapala, D.K.: Quantitative interactions of feline leukemia virus and its pseudotype of murine sarcoma virus in cat cells: Requirement for DNA synthesis. J. Gen. Virol. 13: 203-214, 1971.

Nomura, S., Bassin, R.H., Turner, W., Haapala, D.K. and Fischinger, P.J.: Ultraviolet inactivation of Moloney leukaemia virus: Relative target size required for virus replication and rescue of "defective" murine sarcoma virus. J. Gen. Virol. In press.

Peebles, P.T., Haapala, D.K. and Gazdar, A.F.: Deficiency of viral RNA-dependent DNA polymerase in non-infectious virus-like particles released from murine sarcoma virus transformed hamster cells. J. Virol. In press.

Bassin, R.H., Phillips, L.A., Kramer, M.J., Haapala, D.K., Peebles, P.T., Nomura, S. and Fischinger, P.J.: Properties of 3T3 cells transformed by sarcoma virus in the absence of replicating murine leukemia helper virus. In Chieco-Banchi, L. and Dutcher, R.M. (Eds.): Proc. Vth International Symposium on Comparative Leukemia Research. In press.

Serial No. NCI-4826
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: A. The nature, mechanisms, and stability of cell transformation induced by murine sarcoma virus

B. Non-productive infection of mammalian cells with transforming sarcoma genomes: infection across species barriers

C. Antigenic structure-function relationships of purified murine type C virus components

Previous Serial Number: Same

Principal Investigator: Dr. Peter J. Fischinger

Other Investigators:
Dr. Robert H. Bassin
Dr. Daniel Haapala
Dr. Shigeko Nomura
Dr. Paul T. Peebles
Dr. Leo A. Phillips

Cooperating Units:
Inside NIH
Staff, VLLB, NCI

Outside NIH
Dr. Werner Schafer, Max Planck Institut fur Virusforschung, Tubingen, Germany
Dr. Jens Lange, Max Planck Institut
Dr. Dani Bolognesi, Duke University

Man Years:

Total:	2.0
Professional:	1.0
Others:	1.0

Project Description

Objectives:

A. To determine the mode of integration of the various MSV genomes into cellular genetic mechanisms, specifically to enhance the rate of spontaneous reversion from the transformed state.

B. To coat the MSV genome with envelopes from feline leukemia virus (FeLV) to produce a virus with extended host range, specifically to get human cells infected only with the MSV genome non-productively.

C. To isolate specific molecules and substructures of MuLV, to identify their antigen content, and to relate them to some aspects of biological function.

Methods Employed:

A. and B. Standard virological assays of MSV focus formation, of MuLV focus induction in S+L- cells, of viral interference, and of virus antigen induction, are being used. Cloning of cells in microtiter dishes and as soft agar colonies with the usual biochemical modes of particle and protein isolation are employed routinely.

C. Biochemical methods such as column chromatography, velocity, and isopycnic density gradient centrifugation, radioactive tracer studies, preparative and analytical polyacrylamide gel electrophoresis, and the usual methods of extraction for proteins and nucleic acids are being employed.

Major Findings:

A. Cells transformed only with the MSV genome have been found to give revertants from which the murine sarcoma virus could no longer be rescued. Progeny cells derived from single cloned cells could give rise to variants which behaved as normal cells in their morphology and high degree of contact inhibition. Revertants did not produce particles, did not have any reverse transcriptase in the culture supernates, but were able to detect an MSV population which did not register as foci in normal 3T3 cells. Spontaneous back transformation was found but these cells still did not have a rescuable sarcoma genome. The original MSV genome could not be recovered, up to now, from either revertants or back transformants by rescue techniques or chemical induction methods.

B. The feline leukemia virus pseudotype of MSV (MSV(FeLV)) was produced in high titer and with ratios of the sarcoma to leukemia virus ≥ 1. The virus was obtained from cat cells by co-centrifugation and by rescue techniques from S+L- cells. A variety of cat and human cells have been and are being analyzed to gain evidence of single hit by interaction of the MSV transforming unit and a human cell.

C. Various group specific (gs) antigens of MuLV have been isolated and identified immunologically. Known reference antisera were analyzed regarding their reactivity with known MuLV subtypes and their degradation products. A new nomenclature of MuLV antigens was proposed and accepted. A purified hemagglutinin was isolated from MuLV and found to contain both a gs and a type specific antigen (IIgs and IIv) as detected in immuno-diffusion and neutralizing antibody absorption methods. Another highly

purified unknown antigen "X" was also isolated free from other viral components. The interspecies antigen could be obtained at times free from detectable antigen IV (the main gs antigen) reactivity. This series of papers showed for the first time that there were at least four group specific proteins found among MuLV strains.

The availability of characterized antisera allowed accurate antigen identification of other candidate type C virus whose exact nature was not known. The budding virus from S+L- cells was found to contain only the main gs antigen IV and the interspecies antigen V. The other structural components were presumably missing or of a different antigen nature. Neutralization cross absorption studies showed that the type specificity of the S+L- particle was different from either standard MuLV group. Recently some gs antigens were found to be biologically active for MuLV infectivity.

Significance to Biomedical Research and the Program of the Institute:

A. To understand the mechanisms of cell transformation is important; to induce reversion from the transformed to the normal state is to subvert oncogenesis. It is clear that some MSV genomes are more or less stable in their maintenance of transformation. Any procedure or trend which would accelerate the rate of reversion from the already transformed state has obvious potential curative value.

B. The model system of rescuing native viruses has been established for native rat and hamster viruses by the availability of transformed, non-productive, cells. The MSV genome is now available for entry into human cells. An isolation of a human non-producer cell can be followed by chemical induction methods which could elicit the native human virus-coated sarcoma virus.

C. The dissection of the MuLV structure serves as a fundamental model for other mammalian oncogenic viruses with relevance to common structures and functions. New isolates from human sources have engendered controversy regarding the true nature of these altered viruses. Characterized reagents from model systems will readily define whether human, or contaminating known animal leukemia viruses, or interacting hybrids are involved.

Proposed Course:

A. Further analyses of the reversion process with testing of suggestive chemicals to increase the reversion rate.

B. The isolation of a human non-producer cell.

C. To analyze other structure-function relationships of MuLV antigens and hopefully a reconstitution of biological activity from component parts.

Honors and Awards:

Dr. Fischinger presented two seminars to the Max Planck Institut fur Virusforschung in Tubingen on murine sarcoma virus transformation of mammalian cells. July 3 and 10, 1971.

Dr. Fischinger was the guest speaker in the F. Delafield Lecture series at Columbia University, New York on March 3, 1972. "Antigenic Structure-Function Relationships in Murine Leukemia Viruses".

Publications:

Fischinger, P. J. and Moore, C. O.: The formation and nature of foci induced by a modified sarcoma virus in human cells. J. Gen. Virol. 12: 59-63, 1971.

Fischinger, P. J. and Haapala, D. K.: Quantitative interactions of feline leukemia virus and its pseudotype of murine sarcoma virus in cat cells: Requirement for DNA synthesis. J. Gen. Virol. 13: 203-214, 1971.

Fischinger, P. J., Schafer, W., and Seifert, E.: Detection of some murine leukemia virus antigens in virus particles derived from 3T3 cells transformed only by murine sarcoma virus. Virology 47: 229-235, 1972.

Schafer, W., Fischinger, P. J., Lange, J., and Pister, L.: Properties of mouse leukemia viruses. I. Characterization of various antisera and serological identification of viral components. Virology 47: 197-209, 1972.

Schafer, W., Lange, J., Fischinger, P. J., Frank, H., Bolognesi, D. P., and Pister, L.: Properties of mouse leukemia viruses. II. Isolation of viral components. Virology 47: 210-228, 1972.

Serial No. NCI-4827
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The isolation, purification, and characterization of viral and tumor antigens

Previous Serial Number: Same

Principal Investigator: Dr. Vincent W. Hollis, Jr.

Other Investigators: Dr. Tadao Aoki

Cooperating Units: Inside NIH
Dr. Brenda Gerwin, VLLB, NCI
Dr. Daniel Haapala, VLLB, NCI
Dr. Leo A. Phillips, VLLB, NCI

Outside NIH
Dr. Kyu M. Lee, Cornell University, Ithaca, New York
Dr. Norman Weliky, TRW Systems, Los Angeles, Calif.

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

The basic aims of this project are to isolate and purify the various antigenic components of the murine leukemia viruses, and later other viruses and tumors, (a) to determine the amino acid content and sequence of these antigenic components; (b) to test these antigenic components for their ability to cross react with other tumor viruses; (c) to determine which of the purified fractions elicits the production of protective antibody when used as a vaccine; and (d) to develop new and/or adapt existing biochemical methods for the isolation and characterization of viruses or specific antigens associated with neoplasia.

Methods Employed:

Standard techniques of differential centrifugation, molecular-sieve (gel) and ion-exchange chromatography, isoelectric focusing, and disc-gel electrophoresis were used for the isolation and purification of the viral enzyme and antigens. The enzyme, i.e., RNA-directed DNA polymerase is identified by a standard assay procedure. Procedures for the direct conjugation of ferritin to anti-mouse γ-globulin with meta-xylylene diisocyanate, for use in immunoelectron microscopy. We are developing a method for the chemical conjugation of Southern Bean Mosaic virus to antibodies.

Major Findings:

Data on the purification of the RNA-directed DNA polymerase from Rauscher leukemia virus seems to indicate that the purified enzyme can be broken down into smaller units which have enzyme activity.

From tissues of leukemic AKR mice, a strain which develops spontaneous leukemia without a visible viremia, we have isolated an enzyme which has characteristics of the viral RNA-directed DNA polymerase.

Preliminary experiments with immunoelectron microscopy indicate that the ferritin-conjugation procedure can be adapted for our use.

Significance to Biomedical Research and the Program of the Institute:

The isolation, purification, and chemical characterization of the antigens of a murine leukemia virus contribute to the elucidation of the structure of individual viruses and will assist in determining definite inter-relationships among other murine leukemia viruses. These and other procedures, when applied to the control or prevention of human leukemia may aid in the development of methods for immunological detection of this disease.

Proposed Course:

Studies will continue in an effort to elucidate the chemistry of viruses that induce leukemia and solid neoplasms in the murine and feline species. More specifically, it is planned: (1) to continue the studies on the isolation, purification, and chemical identification of viral and tumor antigens; (2) to begin studies concerned with the isolation, purification, and characterization of the antigens of murine mammary tumor, and feline leukemia viruses; and (3) to continue studies utilizing immuno-ferritin procedures to examine viral and tumor surface antigens; and (4) to elute antibody from AKR tissues to check for their anti-polymerase activity.

Honors and Awards:

None.

Publications:

Sibal, L.R., Hollis, V.W., Jr., and Fink, M.A.: Detection of antigenic subunits of a murine leukemia virus by passive hemagglutination-inhibition. J. Immun. 106: 1050-1055, 1971.

Serial No. NCI-4828
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Immunological studies in animal and human tumors, to include in vitro growth, characterization, diagnosis, therapy and prophylaxis of viral neoplasia.

Previous Serial Number: Same

Principal Investigator: Dr. Ernest J. Plata

Other Investigators: Dr. Tadao Aoki

Cooperating Units: Inside NIH
Dr. Brenda I. Gerwin, VLLB, NCI
Dr. Robert Bassin, VLLB, NCI
Dr. Harish C. Chopra, VB, NCI
Dr. Ronald B. Herberman, LCBGH, NCI

Outside NIH
Dr. Donald Mashburn, Washington Sanitarium & Hospital, Takoma Park, Maryland
Dr. Paul Terasaki, University of California, Los Angeles, California
Dr. F. Pflugy, Malcolm Grow Memorial Hospital, Andrews Air Force Base, Camp Springs, Maryland
Dr. James McCoy, Bionetics Research, Inc., Bethesda, Maryland.
Dr. B. Y. Wat, Loma Linda University, Loma Linda, California

Man Years:

Total:	3.5
Professional:	1.0
Other:	2.5

Project Description

Objectives:

A. To develop new, or to adapt current methods for the detection, identification, and characterization of viruses or specific antigens associated with neoplasia.

B. To study the humoral and cellular immune response of the host to its own and homologous tumors.

C. To explore applicable immunolgical models for improved diagnosis, improved therapy, or possible prophylaxis of cancer.

Methods Employed:

A. Detection of viruses or antigens associated with neoplasia.

1. Murine mammary tumor studies. A C3H mouse transplantable mammary tumor (MT) producing abundant virus (MTV) and tumor associated antigens was used as a model system. Young adult mice of MTV+ and MTV- strains were immunized with C3H-MT by the most appropriate protocols. They produced antibodies and immune lymphocytes that were used to detect the presence, incidence, and character of tumor specific antigens in mice free of tumors or those bearing transplanted or spontaneous mammary tumors. The tests used included indirect immunofluorescence with viable cells, Cr^{51} lymphocyte cytotoxicity, plating inhibition, and colony inhibition tests.

2. Human breast tumor studies.

a. Tissue culture. Phase one of the effort was the establishment of cultures and cell lines from human breast tumors suitable for more advanced studies. This part has now been essentially completed. A consecutive series of 32 benign and 68 malignant human breast tumors were received. The tumors were maintained in wet ice for the 2 to 6 hours that elapsed from incision through processing in the laboratory. Tissues were prepared for culture by mincing, trituration, and treatment with trypsin or pancreatic enzymes to separate viable cells. A modification of the "spill technique" of Ozello and Las Forgues was used. Growth was obtained in 26/32 benign and 59/68 malignant tumors. Generally from 2 to 5 morphologically different cell types could be observed on primary plating. These included the ubiquitous fibroblasts (several types), epithelioid cells and lymphoid appearing cells growing in suspension cultures. Primary cultures and their subsequent 5 passages were minutiously screened for colonies of cells that (1) were clearly epithelioid, (2) were light refractive and birefringent, (3) were not contact inhibited and (4) had multiple cells with mitotic figures. These characteristics plus visible abnormalities in mitotic formations were considered suggestive in vitro indicators of malignancy. Colonies fitting this description were rare and when found were immediately isolated and transferred with micropipettes to new flasks. From this series of 100 tumors, thus far, two purely epithelioid cell lines have been established: one, HBT-3 currently in passage 40-50, was developed and studied in collaboration with Dr. R. Bassin (see report NCI-4848); the second, HT-39 is currently

in passage 30-40. Two other similar epithelial lines appear equally promising at passages 8 and 13 repectively. Each line was derived from a different patient with malignant tumor. No cells of this type were ever seen in non-malignant tumors. In all four cases a companion fibroblast line is being carried which was isolated from the same tumor. Thirty-two cell lines not meeting the criteria of growth listed above were stored in liquid N_2 at passage 4-6. Six lines of fibroblasts are now in passage 10-20. Twelve tumors are still in culture and 46 tumors ceased to grow in cultures between passages 1 and 15.

b. Electron microscopy. Fresh tumors, cells from primary cultures and those from every 5th serial passage were examined by electron microscopy to detect any virus-like particles present. No such particles were seen by Dr. H. Chopra.

c. Oncogenicity. Cells from the HBT-3 and HT-39 epithelial breast lines were tested for their capacity to form tumors in heterologous hosts. 10^6 to 10^9 cells were inoculated into (1) newborn mice and rats, (2) hamsters' cheek-pouch, (3) cortisone treated mice, rats, and hamsters, and (4) intrauterine inoculation of fetal rats. Granulomatous growth developed in about 1/3 of the animals tested in the first two weeks. The growth regressed and disappeared within 4 weeks. No further sign of tumorgenicity was seen.

d. Species certification. Cells from the two breast tumor lines were identified to be of human origin by karyology, HL-A typing, immunofluorescence with species specific standard antisera (Tissue Culture Association) and G6PD and LDH enzyme patterns (American Type Culture Collection).

e. Biochemical survey for viruses. In collaboration with Dr. B. Gerwin (see report NCI-4846) supernatants from HT-39 cell cultures, with or without added RNA or DNA incorporation radioactive labels, were concentrated, banded in sucrose gradients and fractionated. Selected fractions were tested for the presence of viruses by (1) label accumulation, (2) electron microscopy, and (3) reverse transcriptase enzymatic activity. No indication of viral activity was noted.

B. To study the humoral and cellular immune response of the host to autologous or heterologous tumors. One hundred and eighty four sera were collected from patients with malignant breast tumors, non-malignant breast diseases, other tumors, and normal people. These sera were tested by indirect immunofluorescence technique on acetone fixed HT-39 cells. The same sera were tested by complement fixation in a test using crude membrane extracts obtained from HBT-3 cells. No significant levels or patterns of antibody distribution were detected by these tests. Current tests for patient immunity against cultured breast tumor cells include Cr^{51} release cytotoxicity, indirect fluorescence with viable cells, plating and colony inhibition techniques.

Major Findings:

A. At least one MTV-associated soluble antigen was found in the plasma from MTV+ strain mice. This soluble antigen has a specificity different from G(Gross) leukemia virus-associated antigens.

B. Development of tissue culture techniques that led to the establishment of at least three well characterized epithelioid cell lines and companion fibroblast cell lines derived from the same malignant human breast tumors.

Significance to Biomedical Research and the Program of the Institute.

The research approach outlined here is specifically oriented to perform experiments in vitro using animal model systems as well as materials derived from tumor patients. The findings of virus-associated soluble antigens present in the plasma of mammary tumor bearing mice are significant because while soluble antigen has been previously reported from leukemia, such antigens have not been demonstrated before for any solid tumor. This approach is yielding results which may be directly applicable to the solution of problems of the diagnosis, etiology, treatment, or prevention of human malignancy. The experiments performed fit well within the program of Etiology, Viral Oncology, and the SVCP of this Institute.

Proposed Course:

The goals of these projects for the next fiscal year are:

A. To isolate and identify one or more tumor specific or viral antigen from human breast tumors. To produce antiserum to at least one antigen that may permit more rapid identification of good prospective human specimens for study. To begin characterization of at least one isolated specific antigen.

B. To isolate, characterize and describe the pathogenesis of at least one tumor specific antigen in the murine mammary tumor model system leading to a better understanding of its relationship to the host's immune mechanism.

C. To continue to study the question of whether animal oncogenic viruses play a discernible role in human leukemia or breast cancer.

Honors and Awards:

None

Publications:

Fink, M.A., Sibal, L.R., and Plata, E.J.: Serological detection of feline leukemia virus antigens or antibodies. J. Amer. Vet. Med. Ass. 158: 1070-1075, 1971.

Serial No. NCI-4831
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Viral Leukemia Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on murine leukemia

Previous Serial Number: Same

Principal Investigator: Dr. Ruth Merwin

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

To continue studies on reticulum cell neoplasms of the type B in Balb/c mice with particular attention to the behavior of the tumors when transplanted.

Methods Employed:

Standard methods of tumor transplantation and histological identification were used.

Major Findings:

A. When spontaneous reticulum cell neoplasms were transplanted into mice a few days to 1-1/2 months of age and into mice 8 to 13 months of age, the incidence of takes was much higher (75% vs. 16%) in the older mice. This finding suggests that after the transplantation of tumors from the old primary host into young recipients the tumors may remain quiescent until conditions change due to aging of the host.

B. An extract prepared from a tumor that had been transplanted for many generations yielded several tumors after long latent periods. The material was tested to determine whether an effective leukemogenic agent was present.

Tumors arose in inoculated mice only in the mice receiving unfiltered materials, where cells may also have been present.

C. Tumor cells were stored in intraperitoneally-placed diffusion chambers which had walls that tissue fluids but not cells could pass through. Cells retrieved from two of these chambers after 8 to 9 months produced local growths. These cells were altered immunologically so they were either less antigenic or less responsive to immunity in the host. Thus, when mice were pretreated with irradiated cells the challenge cells grew. When cells from a line of passage of the tumor not stored in diffusion chambers were used for pretreatment and challenge, the cells did not grow.

D. All reticulum cell neoplasms type B are generalized in their mode of growth in the primary hosts but show varying degrees of localness under various experimental conditions when transplanted subcutaneously. Local growth developed only after a number of transplant generations, the number varying with different tumors. Tumors arising after long latent periods of 6 to 10 months produced very local growths.

Significance to Biomedical Research and the Program of the Institute:

A. The reticulum cell neoplasms type B in Balb/c mice provide a valuable model to the understanding of the related human tumor, Hodgkin's disease.

B. The type of remission and relapse found in some human leukemias and lymphomas appear to be rare in mice, so an animal model is not presently available for the study of the host control of tumor cell growth. The long latent periods after transplantation of reticulum cell tumors in mice may provide such a model.

C. Enhancement of tumor growth by humoral antibody may be one of the causes of the escape of tumors from host controls. The local or generalized mode of growth of reticular tissue neoplasms in mice probably involves enhancement and it seems to provide an easily observable and sensitive indicator of immunological conditions created by the presence of the tumor. The conditions affecting local and general growth should be studied as a possible source of information that would help in understanding the way in which enhancement develops and affects tumor growth.

Proposed Course:

To continue the study of the conditions affecting the growth of reticular tissue neoplasms in Balb/c mice.

Honors and Awards:

None

Publications:

None

Serial No. NCI- 4834
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: (A) Pathogenesis of Herpesvirus Saimiri.

(B) Murine Rauscher Leukemia Virus Propagated in Human Cells.

Previous Serial Number: Same

Principal Investigator: Dr. Dharam V. Ablashi

Other Investigators: None

Cooperating Units: Inside NIH
Dr. U. Heine, Ultrastructure Studies Unit, ET, NCI
Dr. R. H. Adamson, Pharmacology and Experimental Therapeutics Section, Chemotherapy, NCI
Dr. H. C. Chopra, VBB, NCI

Outside NIH
Dr. W. F. Loeb, Bionetics Research Laboratories, Kensington, Maryland
Dr. S. S. Yang, Bionetics Research Laboratories, Bethesda, Maryland
Dr. D. Dalgard, Hazleton Laboratories, Virginia

Man Years:

Total:	3.0
Professional:	1.0
Other:	2.0

Project Description

Objectives:

A. Due to the oncogenic nature of Herpesvirus saimiri (HVS) in primates, studies were directed to understand the virologic, immunologic, pathologic, and chemotherapeutic aspects of

HVS in vitro and in vivo systems.

B. To activate the defective viral genome and to get higher yields of infectious Rauscher leukemia virus (RLV) from human embryonic kidney (HEK) cells by use of BrdU.

Methods Employed:

A. Primary and continuous primate and human cell lines were used for virus propagation and biological assays. The electron microscopy (EM) was also employed in a search for virus particles. Both owl and squirrel monkeys were used for in vivo experiments.

B. A continuous HEK cell line carrying either the RLV genome or the RLV were used. Uninfected HEK cells were used for virus assay.

Major Findings:

A. In vivo. All owl monkeys (30) inoculated with HVS over a period of 1 1/2 years consistently developed malignant lymphoma with lymphocytic leukemia. However, in the majority of animals, two injections of virus were needed. In contrast, 15 old world HVS inoculated monkeys failed to develop any disease.

Lymphoma cells from an HVS-infected owl monkey induced lymphoma without frank leukemia in an adult owl monkey after a period of 9 1/2 months, establishing the longest incubation period thus far reported with HVS-induced tumors.

All owl monkeys exhibited the HVS genome in the peripheral blood and in the tumors, but failed to demonstrate free virus particles by EM. These monkeys also developed higher titer neutralizing, fluorescent, and complement fixing antibodies. Noninoculated squirrel monkeys, the natural host of HVS, were found to possess low titer antibodies ($\geq$80%) to HVS. No pre-existing HVS antibody has been found in other primates (3 old world and 3 new world). Simultaneous persistence of the HVS antibody and genome was detected in squirrel monkeys over a period of 18 months, suggesting a possible role of humoral antibody in prevention of viral pathogenesis.

In vitro HVS was found to be very stable at 4°C. over a period of 2 months. The virus was also found to be cell associated. A long term culture of lymphoma cells from an HVS infected owl monkey provided a useful tool for molecular biology studies.

HVS was detected only in early passages.

Out of the four anticancer drugs (cytosine arabinoside, adenine arabinoside, tilorone and rifamycin SV) used in vitro for inhibition of HVS multiplication, three were effective at a level not toxic to the cells. Cytosine arabinoside completely blocked the viral synthesis at 24 hours after post HVS infection.

B. Treatment by BrdU of HEK induced higher infectivity (1/2 log to 1 log) titers in cells chronically producing RLV.

Significance to Biomedical Research and the Program of the Institute:

A. Even though oncogenic herpesviruses have been reported from chickens, frogs, and rabbits, HVS is so far the only well documented oncogenic herpesvirus from a primate. This virus provides a model to study the oncogenicity of herpesvirus infection in species more closely related to man and to explore and evaluate the immunotherapy (humoral and cellular immunity) and chemotherapy (anticancer agents) of virus-induced tumors.

B. The treatment of HEK cells infected with a murine leukemia virus with BrdU shows that it can increase the virus yields in human cells as well as rodent and chicken cells.

Proposed Course:

A. Immunological and chemotherapeutic aspects of HVS pathogenesis remains to be completed. In vivo experiments will be conducted to find the site of viral replication and possible role of other virus, e.g., type C in the induction of tumors.

B. Treatment of human cells chronically infected with C-type viruses with chemical inducers such as BrdU may provide a means of increasing low level expression of endogenous C-type virus information in human cells.

Honors and Awards:

Presented seminars on Herpesvirus saimiri and Rauscher leukemia virus propagated in human cells at:

1. University of Rhode Island, Kingston, Rhode Island

2. Rhode Island Hospital, Providence, Rhode Island

3. NIH - (January 4, 1972). Peripheral Lymphocyte Motility Associated with HVS-Induced Leukemia and Lymphoma.

The following papers have been accepted for presentation at 1971 meetings:

1. Ablashi, D.V., Armstrong, G.R., Heine, U., and Adamson, R.G.: Establishment of a cell culture from an owl monkey tumor induced by Herpesvirus saimiri (HVS). Amer. Assoc. Cancer Res. May 4-6, 1972, Boston, Mass.

2. Ablashi, D.V., Loeb, W.F., Armstrong, G.R., Yang, S.S., Valerio, M.G., and Adamson, R.H.: Oncogenicity of Herpesvirus saimiri induced lymphoma and the DNA polymerases of the lymphoma and the DNA polymerases of the lymphoma derived cell line and Herpesvirus saimiri. 3rd Conference on Exper. Med. & Surg. in Primates, June 21-23, 1972, Lyon, France.

Publications:

Heine, U., Kondratick, J., Ablashi, D.V., Armstrong, G.R., and Dalton, A.J.: Ultrastructure and biologic properties of a cytomegalo virus rescued from human paraganglioma. Cancer Research 31: 542-549, 1971.

Ablashi, D.V., Armstrong, G.R., Heine, U., and Manaker, R.A.: Propagation of Herpesvirus saimiri in human cells. J. Nat. Cancer Inst. 47: 241-244, 1971.

Heine, U.I., Ablashi, D.V., and Armstrong, G.R.: Morphological studies in Herpesvirus saimiri in subhuman and human cell cultures. Cancer Research 31: 1019-1029, 1971.

Ablashi, D.V., Loeb, W.F., Valerio, M.G., Adamson, R.H., Armstrong, G.R., Bennett, D.G., and Heine, U.: Malignant lymphoma with lymphocytic leukemia in owl monkeys induced by Herpesvirus saimiri. J. Nat. Cancer Inst. 47: 837-855, 1971.

Adamson, R.H., Ablashi, D.V., Armstrong, G.R., and Ellmore, N.W.: Effect of cytosine arabinoside, adenine arabinoside, tilorone and rifamycin SV on multiplication of Herpesvirus saimiri in vitro. Antimicrobial Agents and Chemotherapy 1: 82-83, 1972.

Ablashi, D.V., Turner, W., Armstrong, G.R., and Bass, L.R.: Characterization of murine Rauscher leukemia virus propagated in human cells. J. Nat. Cancer Inst. 48: 615-621, 1972.

Ablashi, D.V., Chopra, H.C., and Armstrong, G.R.: A cytomegalovirus isolated from an owl monkey. Lab. Animal Sci. 22: 190-195, 1972.

Ablashi, D.V., Armstrong, G.R., and Blackham, E.A.: Biological characteristics of Herpesvirus saimiri cultured in various subhuman cells. Amer. J. Vet. Res. In press.

Serial No. NCI-4834

Chopra, H.C., Lloyd, B.J., Ablashi, D.V., and Armstrong, G.R.: Morphologic studies of a cytomegalovirus isolated from an owl monkey. J. Nat. Cancer Inst. In press.

Serial No. NCI-4835
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: A. Studies of cell lines infected with murine sarcoma virus in absence of MuLV: rescue phenomenon and evidence for production of a defective virus-like particle.

B. Investigation of the biological infectivity and polymerase activity of a hamster-derived MSV

C. Virus search in pediatric solid tumors

Previous Serial Number: Same

Principal Investigator: Dr. Paul T. Peebles

Other Investigators: Dr. Adi Gazdar
Dr. Robert H. Bassin
Dr. Peter J. Fischinger
Dr. Daniel Haapala
Dr. Shigeko Nomura
Dr. Leo A. Phillips

Cooperating Units: Inside NIH:
Staff of the VLLB, NCI

Outside NIH:
None

Man Years:

Total:	2.3
Professional:	1.0
Other:	1.3

Project Description

Objectives:

A. To study rescue phenomena from cell lines infected with murine sarcoma virus (MSV) in the absence of murine leukemia virus (MuLV) and to characterize defective virus-like particles produced from these lines and clonal cell lines of non-transformed revertants.

B. To investigate the biological infectivity and RNA-dependent DNA polymerase activity of a mammalian MSV.

C. To search for viruses in pediatric solid tumors.

Methods Employed:

A. Sarcoma and leukemia virus production in cell culture were measured by known tissue culture assays, tritiated uridine labelling, immunological tests, and electron microscopy. The requirement for DNA synthesis in virus propagation was determined by assaying virus production with and without DNA synthesis inhibitors. Virus production from revertant cell lines was measured by reverse transcriptase activity in their supernatant fluids.

B. Defective MSV was concentrated from supernatant fluids by double sucrose density purification. Viral reverse transcriptase was assayed for with and without synthetic RNA-DNA templates.

C. The above techniques employed in the investigation of MSV and MuLV have been utilized in an attempt to find evidence for a viral etiology in pediatric solid tumors.

Major Findings:

A. The isolation of clonal cell lines infected with MSV in the absence of detectable MuLV provided the opportunity to study the defectiveness of MSV, and the development of a quantitative MuLV assay permitted the study of MuLV kinetics during MSV rescue.

The nature of murine sarcoma virus (MSV) "defectiveness" was investigated by employing an MSV-transformed mouse 3T3 cell line which releases non-infectious virus-like particles. Rescue kinetics of MSV, observed after murine leukemia virus (MuLV) superinfection of these "sarcoma-positive leukemia-negative (S+L-)" mouse 3T3 cells, consisted of a 9- to 12-hour eclipse period followed by simultaneous release of both MSV and MuLV with no evidence for release of infectious MSV prior to the production of progeny MuLV. Addition of thymidine to the growth medium of MuLV-superinfected S+L- cells at a concentration suppressing deoxyribonucleic acid synthesis inhibited the replication of MuLV and the rescue of MSV. MSV production closely paralleled MuLV replication under a variety of experimental conditions. These results suggest that replication of MuLV is required for the rescue of infectious MSV from S+L- cells and that one (or more) factor, produced late in the MuLV replicative cycle, is utilized by both viruses during virion assembly. During the course of these experiments, virus stocks were recovered which contained infectious MSV in apparent excess over MuLV. These stocks were used for generating new S+L- cell lines by simple end point dilution procedures.

In order to further characterize these S+L- cells, the original cloned lines were studied and found to be releasing a C-type virus-like particle which was seen on EM and also at a 1.16 buoyant density in a sucrose gradient. This particle lacks biological infectivity on conventional cell lines, even after co-sedimentation with MuLV.

B. The MSV hamster derived tumor cell lines HTG and HTG2 produce large quantities of non-infectious type C particles with a buoyant density of 1.16 and are antigenically related to the original murine virus. Competent focus forming MSV could be isolated from these lines when they were co-cultivated with mouse embryo fibroblasts and superinfected with MuLV. Virions from hamster cells transformed by the new strain of murine sarcoma virus appear to represent a form of MSV deficient in one or more of the viral components necessary for infectivity. Both the non-infectious virions and the sarcoma-virus-transformed cells, which have no detectable replicating leukemia helper virus of either murine or hamster origin, are deficient in viral-type RNA-dependent DNA polymerase activity (i.e., reverse transcriptase activity stimulated by the synthetic RNA-DNA template polyriboadenylic-oligodeoxythymidylic acid). In comparison, both murine leukemia virus (MuLV) and MuLV-infected cells have abundant viral-type reverse transcriptase activity. It appears, therefore, that the leukemia virus genome may be required for the production of infectious sarcoma virus by contributing information needed for the full expression of functional viral-type reverse transcriptase.

C. Studies are in progress with tumor material from pediatric patients with solid tumors.

Significance to Biomedical Research and the Program of the Institute:

A. The exploration of oncogenic RNA viruses, their defectiveness, and the variance between their capacity to replicate and their capacity to transform has great implications in that their existence makes probable the viral etiology for human cancer.

B. When human oncogenic viruses are isolated they are likely to function in a manner analogous to the mammalian oncogenic viruses. The inability to find viral reverse transcriptase in sarcoma transformed cells indicates that, if human tumor cells are found to be deficient in this enzyme, the possible presence of defective viral genomes is not ruled out.

C. The ability to quickly produce S+L- cells may serve as a method for producing a human cell line with a sarcoma genome that would release detectable virus when superinfected with a human leukemia virus, and a cell line of this type could be used for detecting human leukemia viruses that may possibly be present in pediatric solid tumors.

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Proposed Course:

A. We are attempting to further characterize the MSV defectiveness in S+L- cells in comparison to other MSV phenotypes, i.e., "NP" cells and S+L- virus.

B. We are also involved in attempting to demonstrate the genetic stability of the S+L- phenotype and thus furthering evidence for a new and distinct MSV genotype.

C. We are also continuing to attempt to demonstrate a viral etiology in pediatric solid tumors.

Honors and Awards:

None

Publications:

Bassin, R. H., Phillips, L. A., Kramer, M. J., Haapala, D. K., Peebles, P. T., Nomura, S., and Fischinger, P. J.: Properties of 3T3 cells transformed by sarcoma virus in the absence of replicating murine leukemia helper virus. In Chieco-Banchi, L. and Dutcher, R. M. (Eds.): Proc. Vth International Symposium on Comparative Leukemia Research, Padua, Italy. In press.

Bassin, R. H., Phillips, L. A., Kramer, M. J., Haapala, D. K., Peebles, P. T., Nomura, S., and Fischinger, P. J.: Transformation of mouse 3T3 cells by murine sarcoma virus: Release of virus-like particles in the absence of replicating murine leukemia helper virus. Proc. Nat. Acad. Sci. U.S.A. 68: 1520-1524, 1971.

Gazdar, A. F., Phillips, L. A., Sarma, P. S., Peebles, P. T., and Chopra, H. C.: Presence of sarcoma genome in a "non-infectious" mammalian virus. Nature New Biol. 234: 69-72, 1971.

Peebles, P. T., Bassin, R. H., Haapala, D. K., Phillips, L. A., Nomura, S., and Fischinger, P. J.: Rescue of murine sarcoma virus from a sarcoma-positive leukemia-negative cell line: Requirement for replicating leukemia virus. J. Virol. 8: 690-694, 1971.

Peebles, P. T., Haapala, D. K., and Gazdar, A. F.: Deficiency of viral RNA-dependent DNA polymerase in non-infectious virus-like particles released from murine sarcoma virus transformed hamster cells. J. Virol. In press.

Serial No. NCI-4836
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mechanisms of virus-induced transformation.

Previous Serial Number: NCI-4914

Principal Investigator: Dr. Stuart A. Aaronson

Other Investigators: Dr. John R. Stephenson
Dr. George J. Todaro
Dr. Garth Anderson
Dr. Steven R. Tronick
Dr. Raoul E. Benveniste

Cooperating Units: Inside NIH
Dr. Wade P. Parks, VCB, NCI
Dr. Wallace Rowe, LVD, NIAID
Dr. Robert J. Huebner, VCB, NCI
Dr. Malcolm Martin, LBV, NIAID
Dr. Robert H. Bassin, VLLB, NCI
Dr. Tadao A. Aoki, VLLB, NCI
Dr. Peter J. Fischinger, VLLB, NCI
Dr. Edward M. Scolnick, VLLB, NCI
Dr. David M. Livingston, VLLB, NCI

Outside NIH
Dr. David Lytle, Environmental Health Bureau, Rockville, Maryland
Dr. Harvey Dosik, Maimonides Hospital, Brooklyn, New York
Dr. John Kersey, University of Minnesota, Minneapolis, Minnesota
Dr. Raymond Gilden, Flow Laboratories, Rockville, Maryland
Dr. Leonard Hayflick, Stanford University, Stanford, California
Naval Biological Laboratory, Oakland, California

Man Years:

Total:	1.1
Professional:	1.0
Other:	0.1

Project Description

Objectives:

1. To study the mechanisms of action of RNA and DNA-containing tumor viruses.

2. To apply knowledge gained from experimental systems to search for a viral etiology to human neoplasia and to develop rational approaches to prevention and treatment of human tumors.

Methods Employed:

Standard cell culture procedures of this laboratory. Immunological and biochemical techniques such as enzymology and nucleic acid homology studies.

Major Findings:

1. Continuous lines of Balb/c and NIH/Swiss embryo cells have been developed which are very useful for study of normal growth control mechanisms and the effects of RNA and DNA tumor viruses on growth regulation.

2. A genetic approach to the study of RNA tumor viruses has led to the development of rapid methods for screening large numbers of cultures for temperature sensitive (ts) mutants. Ts mutants of both murine leukemia (MuLV) and sarcoma virus (MSV) have been isolated, and some have been partially characterized.

3. Sarcoma virus transformed cells have been isolated which show normal cell morphology but contain the typical sarcoma viral genome. These cellular mutants are currently being studied to determine the cellular functions involved in the expression of transformation.

4. C-type RNA viruses have been found to be inducible from numerous clonal lines of Balb/c embryo cells by treatment with chemical agents such as BrdU. These studies demonstrate that all Balb/c cells contain a latent C-type viral genome in a genically stable form. In addition, MSV-transformed nonproducer mouse and rat cells isolated in this laboratory can be activated by these same chemicals to produce both MSV and MuLV. An understanding of the mechanisms involved in chemical induction of C-type viruses and genetic factors affecting inducibility and persistence of endogenous viruses are currently being studied.

5. MSV nonproducer cells have been of particular interest because they provide a model system that shows many similarities to the human tumor cell. Nonproducer cells have been found to lack any detectable transplantation antigens in contrast to RNA virus-producing transformed cells which are highly antigenic. Methods have been developed using RNA-DNA hybridization to detect sarcoma virus-specific RNA in the nonproducer cell.

6. The reverse transcriptases of RNA tumor viruses have been extensively investigated. Normal mouse cells have been found to contain two enzymes which can utilize RNA-RNA or RNA-DNA synthetic templates. These enzymes can be distinguished, however, by physical properties, template preferences, and antigenicity from C-type viral polymerase. While viral enzyme can be readily found in virus-producing cells, MSV nonproducer cells lack detectable virus-specific enzyme.

7. Antibodies have been developed which specifically inhibit murine C-type viral polymerases. Antibodies to avian and feline C-type viral enzymes have also been obtained. While murine and feline antipolymerase antibodies inhibit cat, mouse, rat, and hamster C-type enzymes, they do not react with newly isolated primate C-type viruses. Antibodies are now being obtained.

8. Primate isolates of C-type viruses are being characterized biologically, immunologically, and biochemically for use in the search for a viral etiology to human tumors.

9. Human tumor material has been processed, and several lines of tumor cells have been established. These are being extensively investigated for evidence of RNA tumor virus expression using techniques developed from work in model systems.

Significance to Biomedical Research and the Program of the Institute:

The systems that are being intensively investigated have provided a much better understanding of the biology and biochemistry of viral transformation. It is felt that a clear understanding of these phenomena will significantly speed the progress in our search for a viral eitology of human cancer.

Proposed Course:

To continue research already in progress in the following major areas: (1) mechanisms of action of murine sarcoma and murine leukemia viruses; (2) induction of C-type viruses from virus-negative cells; (3) determination of the role of viruses in human neoplasia; and (4) to develop new research areas in model systems that pertain to human disease.

Honors and Awards:

None

Publications:

Aaronson, S.A. and Weaver, C.A.: Characterization of murine sarcoma virus (Kirsten) transformation of mouse and human cells. J. of Gen. Virology 13: 245-252, 1971.

Aaronson, S.A., Todaro, G.J. and Scolnick, E.M.: Induction of murine C-type viruses from clonal lines of virus-free Balb/3T3 cells. Science 174: 157-159, 1971.

Aaronson, S.A.: Chemical induction of focus-forming virus from nonproducer cells transformed by murine sarcoma virus. Proc. Nat. Acad. Sci. U.S.A. 68: 3069-3072, 1971.

Aaronson, S.A., Bassin, R.H. and Weaver, C.: Comparison of murine sarcoma viruses in nonproducer and $S^{+}L^{-}$ transformed cells. J. Virology. In press.

Aaronson, S.A.: Immunologic detection of C-type RNA viral antigens. JNCI Monograph Series. In press.

Aaronson, S.A. and Stephenson, J.R.: Genetic factors involved in C-type RNA virus expression. Membrane, Viruses and Immune Mechanisms in Experimental and Clinical Diseases. In press.

Serial No. NCI-4840
1. Viral Leukemia and Lymphoma Branch, OASDVO, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Non-human primate surgery - development of special techniques and procedures in addition to routine surgery

Previous Serial Numbers: Same

Principal Investigator: Dr. Sergio A. Leiseca

Other Investigators: None

Cooperating Units: Inside NIH
Dr. Roy F. Kinard, VLLB, NCI
Dr. Paul H. Levine, VLLB, NCI
Dr. Robert A. Manaker, VB, NCI
Dr. Jack Gruber, VB, NCI

Outside NIH
Bionetics Research Laboratories
Kensington, Maryland

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description

Objectives:

1. To provide to the National Cancer Institute, and other cooperating institutes, embryonic and fetal material from the primate colony maintained at Bionetics Research Laboratories (BRL).

2. To provide surgical procedures required to maintain and improve the health of primate colony inhabitants.

Methods Employed:

1. Cesarean sections technique performed on primates (primarily by Macaca mulatta).

2. Developed and applied procedures for cannulation of thoracic lymph ducts.

3. Hysterotomy and biopsy of fetal skin and muscle.

4. Intra-uterine surgery in nonhuman primates the 65th day of gestation.

5. Many operative procedures are done to repair various clinical conditions.

Major Findings:

1. C-section techniques are also used for the following: to obtain fetal material for large amounts of primary cell cultures used in NCI; to provide most of the infants for the S.V.C.P. virus inoculation program at the Bionetics Research Laboratories project; to study the effect of carcinogenic materials on fetuses inoculated in utero; to provide through amnio cestesis recovery of drug in the amniotic fluids for the determination of possible inducement of anomalies or tumors, and to salvage all placentae for protein studies in relation to malignancy and birth control.

2. Cannulation of thoracic lymph ducts is used in chimpanzees and rhesus monkeys for the continuous collection of lymph in the study of the malignant tumors.

3. The C-section technique is used to present the fetuses and neonatal animals for immunosuppressive studies and for inoculation of leukemia or other cancerous materials as well as some viruses. Biopsies of fetuses are used for tissue cultures for later infection with cancerous materials and reinjection into the donor.

4. Cannulization of placental fetal vessel and maternal saphenous vein allowed continuous recovery of blood specimens. These specimens are used in investigations of the transfer of drugs, hormones, glucose, etc.

By fetal exsanguination intra-utero blood is obtained for alpha fetal protein studies. This study is a part of the leukemia program.

5. Surgical procedures are routinely performed, repair injuries resulting from accidents that occur in the primate breeding colony. Some of these procedures are:

a. suture and debridement of various wounds, and lacerations of head, body, extremities, tongue, tail

b. drainage of abscess

c. amputations of digits traumatized and tail

d. open reduction of fractures

e. herniorrhaphy-umbilical, vential, or inguinal

f. closure of wounds

g. C-section to obtain fetuses in breech presentation, bleeding due to premature placental seperations, toxemia, protracted labor, prolonged or overdue pregnancy, dead fetus, etc.

h. prolapse of rectus

i. prolapse of vagina and cervix

j. D & C to remove retained decidua following abortion or delivery

k. tumor biopsy

l. laparotomy for ovarian cyst, ruptured uterus, splenomegaly, etc.

Significance to Biomedical Research and the Program of the Institute:

Non-human primates have proven very useful in research on human viral diseases. They are man's closest phylogenetic relative and are the most desirable model system in research projects which cannot utilize humans directly. Much of the work in primate cancer research is dependent on the highest quality surgical procedures for the provision of sterile fetal tissue and healthy young free of pathogenic organisms. Surgical intervention for lymph and other tissues requires great professional skill for maximum animal health and comfort in addition to experimental validity.

Honors and Awards:

None

Proposed Course:

Continue to provide the best possible surgical procedures for primate cancer research.

Publications:

Sibinovic, S., Valerio, D. A., Landon, J. C., and Leiseca, S.: Maintenance of juvenile simians for oncogenic studies. In Goldsmith, E.I. and Moorjankowski, J. (Eds.): Medical Primatology. New York, S. Karger, pp. 912-917, 1971.

Martin, D., Leiseca, S. A., and Darrow, C.: Methods of anesthesia in subhuman primates. Anesthesiology. In press.

Serial No. NCI-4842
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The biochemistry and biophysics of leukemia and sarcoma viruses

Previous Serial Number: Same

Principal Investigator: Dr. Leo Phillips

Other Investigators: Dr. Robert H. Bassin
Dr. Peter J. Fischinger
Dr. Daniel K. Haapala
Dr. Shigeko Nomura
Dr. Paul T. Peebles

Cooperating Units: Inside NIH
Dr. Adi F. Gazdar, VLLB, NCI
Dr. Harish C. Chopra, VBB, NCI

Man Years:

Total:	2.3
Professional:	1.0
Others:	1.3

Project Description

Objectives:

These studies are concerned with the biochemical and biophysical properties of RNA-tumor viruses. Our objectives are as follows:

1. To characterize the nucleic acids and the structural polypeptides of purified virions released from cells which have been transformed by sarcoma viruses.

2. To isolate and characterize the viral specific nucleic acids and polypeptides of cells infected with leukemia and sarcoma viruses.

3. To determine which of the viral specific polypeptides is the RNA-dependent DNA polymerase of the virions.

4. To determine what makes a sarcoma virus either competent or defective.

5. To determine the state of the sarcoma genome in S+L- cells which are not releasing particles; determine whether the genome exists as a plasmid, an episome, or whether it is integrated into the cellular DNA.

6. The ultimate goal of my research is to determine what makes one cell line permissive to oncogenic virus infection and another cell line nonpermissive; therefore, an in vitro protein synthesizing system will be developed to answer this vital question.

Methods Employed:

The focus assay for sarcoma viruses and the helper assay for leukemia viruses are employed for the quantitation of the viruses. [^{3}H]-uridine incorporation in particles with a density of 1.16 g/cm^3, RNA-dependent DNA polymerase assays on supernatant fluids and electron microscopy are utilized to determine if virus is released from transformed cells. For characterization of the viral nucleic acids and polypeptides, standard biochemical and biophysical techniques (analytical and preparative ultracentrifugation, column chromatography, isotopic labeling, polyacrylamide gel electrophoresis, peptide fingerprinting, electrofocusing, in vitro protein synthesis, enzymology, etc.) are being employed.

Major Findings:

When mouse cells are transformed by MSV, some of the transformed cells become "nonproducers", those cells which do not release any detectable virus, and some become "producers", those cells which release virus. The amount of virus released by "producers" varies as indicated by ^{3}H-uridine-5-6 incorporation in the nucleic acids of virions at a density of 1.16 g/cm3 and electron microscopy.

The nucleic acids of both the mouse leukemia virions (S-L+) and the mouse sarcoma virions (S+L-) have S-values of 65, 28, 18, and 4S. Additionally, the sarcoma virions may have a larger species of nucleic acid which contains some DNA and RNA; which could possibly explain sarcoma virions ability to transform cells in the presence of leukemia virions, whereas, leukemia virions alone lack the ability to transform cells.

Significance to Biomedical Research and the Program of the Institute:

These biochemical and biophysical studies of RNA-tumor viruses contain three avenues for obtaining pertinent knowledge for the possible cure of cancer: (1) studies which are being conducted on the viral nucleic acids and structural polypeptides could possibly determine if their mode of replication is by means of transcriptional-translational

complexes, and if so, it may be possible to effect a cure for cancer by specifically blocking the replication of these RNA tumor viruses either at the transcriptional or translational level; (2) studies which are being done to determine the disposition of the viral genome in sarcoma transformed, "nonproducer" cells could possibly enable the excision of the viral genome with interfering with the cellular genome; (3) studies which are being conducted to ascertain the property which makes one cell line permissive, while another cell line is nonpermissive, to infection by RNA tumor viruses will be invaluable in effecting a cure for cancer.

Proposed Course:

These critical biochemical and biophysical studies of RNA tumor viruses will be continued in order of their complexity.

Honors and Awards:

Participant in the 1971 Biology of Cancer Cells Course and Conference at the University of Colorado, Boulder, Colorado, from 21 June to 16 July.

Participant in the 1971 Gordon Research Conference on Animal Cells and Viruses at Tilton, New Hampshire, from 30 August to 3 September.

Publications:

Bassin, R.H., Phillips, L.A., Kramer, M.J., Haapala, D.K., Peebles, P.T., Nomura, S., and Fischinger, P.J.: Transformation of mouse 3T3 cells by murine sarcoma virus: release of virus-like particles in the absence of replicating murine leukemia helper virus. Proc. Nat. Acad. Sci. U.S.A. 68: 1520-1524, 1971.

Gazdar, A.F., Phillips, L.A., Sarma, P.S., Peebles, P.T., and Chopra, H.C.: Presence of sarcoma genome in a "non-infectious" mammalian virus. Nature New Biology 234: 69-72, 1971.

Peebles, P.T., Bassin, R.H., Haapala, D.K., Phillips, L.A., Nomura, S., and Fischinger, P.J.: Rescue of murine sarcoma virus from a sarcoma-positive leukemia-negative cell line: Requirement for replicating leukemia virus. J. Virology 8: 690-694, 1971.

Bassin, R.H., Phillips, L.A., Kramer, M.J., Haapala, D.K., Peebles, P.T., Nomura, S., and Fischinger, P.J.: Properties of 3T3 cells transformed by sarcoma virus in the absence of replicating murine leukemia helper virus. In Chieco-Banchi, L. and Dutcher, R.M. (Eds.): Proc. Vth International Symposium on Comparative Leukemia Research. In press.

Serial No. NCI-4844
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title:

A. Development of new visual marker for immuno-electron microscopy.

B. Analysis of oncogenic RNA C-type virus-associated antigens on the surface of tumor cells and viruses.

C. Classification of Gross soluble antigens (GSA).

D. Activation of Gross leukemia viral genome by lymphocytic choriomeningitis (LCM) virus infection in vitro and in vivo.

E. Antigenic modulation of cell surface and intracellular antigens.

F. Absence of absolute immunological tolerance of AKR mice to Gross leukemia virus-associated antigens.

Previous Serial Number: None

Principal Investigator: Dr. Tadao Aoki

Other Investigators: Dr. Brenda Gerwin
Dr. Vincent W. Hollis
Dr. Ernest J. Plata

Cooperating Units:

Inside NIH
Dr. Ronald B. Herberman, LCBGH, NCI

Outside NIH
Dr. Lloyd J. Old, Sloan-Kettering Institute for Cancer Research, New York, New York
Dr. Edward A. Boyse, Sloan-Kettering Institute for Cancer Research, New York, New York
Dr. Christopher W. Stackpole, Sloan-Kettering Institute for Cancer Research, New York, New York
Dr. Etienne deHarven, Sloan-Kettering Institute for Cancer Research, New York, New York

Outside NIH (continued)
Dr. Harry A. Wood, Boyce Thompson Institute for Plant Research, Yonkers, New York
Dr. Michael B. A. Oldstone, Scripps Clinic and Research Foundation, LaJolla, California
Dr. Frank J. Dixon, Scripps Clinic and Research Foundation, LaJolla, California
Dr. Robert C. Nowinski, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin
Dr. James McCoy, Bionetics Research, Inc., Bethesda, Maryland

Man Years:

Total:	2.3
Professional:	1.8
Other:	1.5

Project Description

Objectives:

A. To develop new visual markers for immuno-electron microscopy in order to examine the relationship among different specific antigens on the surface of cells and viruses.

B. To detect different oncorna virus-associated antigens on the surface of tumor cells and viruses for clarification of the diagnosis, treatment, and etiology of virus-induced tumors.

C. To determine different specific soluble antigens in the plasma of normal and tumor-bearing animals as a model system for human problems.

D., E. & F. To solve problems of tumor growth by the demonstration of activation- or suppression-mechanisms of tumor specific cell surface antigens in virus-induced tumors.

Methods Employed:

A. thru F. In addition to classical immunological methods (i.e., cytotoxic tests, immunofluorescence tests, absorption tests, precipitation techniques, and studies of tumor rejection), immunochemical methods and immuno-electron microscopy have been used to study these problems at molecular levels. We have attempted to apply results obtained in the various animal model systems to human systems in tumor patients as well as tissue cultures.

Major Findings:

A. In addition to ferritin and southern bean mosaic virus, tobacco mosaic virus (TMV) with hybridantibody anti-γG/anti-marker has been established as another visual marker for immuno-electron microscopy. TMV fragmented by treatment with RNase at high pH is a third useful marker. Its shape and specificity are clearly distinguishable from the other two markers. Thus, a new method to examine the relationship among different antigenic sites on the surface of the same cells or viruses is now available for conventional electron microscopy. Furthermore, untreated, full length, TMV may be used to visualize antigen distribution on the cell surface by scanning electron microscopy at relatively low resolution.

B. By using various serological methods, especially immuno-electron microscopy, we can recognize several G(Gross) antigens on the surface of the Gross leukemia cells, e.g., GCSAa,b,IX, and type-specific and group-specific GVEA. Furthermore, it has been clarified that during virus budding G murine leukemia viruses (GMuLV) acquire some alloantigens, $H\text{-}2^k$ and Θ-C3H, which originally existed on the cell surface.

BALB/c mineral oil-induced murine myeloma cells produce many extracellular C-type viruses. These viruses contain type-specific envelope antigen(s) xVEA and an envelope antigen common to one of the Gross leukemia virus envelope antigens. The former is different from Gross leukemia virus type-specific envelope antigen(s) GVEA, and may induce PC-1 antigen which has been considered an alloantigen, for the following reasons: (1) PC-1 antigen positive strains of mice, e.g., BALB/c, produce natural antibody to PC-1 antigen. This antibody reacts with PC-antigen on the surface of BALB/c myeloma cells and extracellular C-type viruses. (2) PC-1 antigen negative strains of mice, e.g., C57BL/6, in contact with PC-1 antigen positive mice also occasionally produce such an antibody.

C. GSA has been originally determined using the G-typing mouse antiserum which can recognize only GCSAa. By further analysis in combination with the G-typing rat antiserum, however, at least two major groups of GSA have been found in leukemic mice as well as normal G^+ mice.

D. When G^+ and apparently G^- cells or mice were infected with cloned LCM viruses, they synthesized GCSA but so far no virus particles have been detected. These findings suggest that LCM viruses activate G viral genome to induce GMuLV-associated antigens.

E. When Gross leukemia E♂G2 induced in C57BL/6 mice by Passage A Gross virus was transplanted into the syngeneic mice immunized with transplanted AKR spontaneous leukemia K36, tumor growth was slowed and the cell surface antigen GCSAa disappeared. By reappearance of this antigen to its

original level after this E♂G2 was retransplanted into normal C57BL/6 mice, a kind of antigenic modulation was also proven to occur in this system. Since C57BL/6 mice can recognize only GCSAa but not GVEA, Gross leukemia virus (GMuLV) production was not influenced at all in this experiment.

In addition, the intracellular antigens of two established human malignant cell lines, African Burkitt's lymphoma P3J and malignant melanoma SK-Mel1, showed a similar antigenic modulation in vitro. Although the exact mechanisms are not known, this phenomenon may be reflected in the cell surface antigen-antibody reaction.

F. For a long period of time AKR mice have been considered to be immunologically tolerant to GMuLV, since they are G antigen positive during their entire life span. It has become evident, however, that the kidney-eluate of untreated AKR mice contains at least two different antibodies to intraviral G antigen(s) and GCSA, although no free antibodies are detected in the body fluid. These findings indicate that there is no absolute immunological tolerance in the Gross leukemia system.

Significance to Biomedical Research and the Program of the Institute:

A. and B. By using different markers for immuno-electron microscopy, we can understand what kinds of oncogenic virus-associated antigens exist on the surface of cells and viruses, their spatial relationship, and which antigens play important roles in tumor suppression or in virus neutralization. In fact, the G-typing rat serum can recognize major transplantation Gross leukemia specific cell surface antigens and GMuLV neutralization antigens. By contrast, the G-typing mouse serum can recognize only a minor transplantation Gross leukemia specific cell surface antigen. The relationship between the spatial distributions of these two different G antigen groups has been partially visualized by immuno-electron microscopy with different markers.

It is of great interest that several strains of mice other than C57BL/6 mice, which are donors of the G-typing mouse serum, can produce natural antibody against murine C-type viral envelope antigens. Further analysis will provide us with a deeper understanding of the etiology of tumors, and indicate possible approaches to the solutions of many problems of human malignant diseases from an immunological point of view.

The finding that oncorna viruses acquire some cell surface alloantigens during budding leads us to examine carefully whether the antiserum used may contain antibody to such alloantigens besides specific antibody to oncorna virus-associated antigens. For the production of vaccine to oncorna virus, these findings will aid in approaching the problem of purifying the specific antibody.

The fact that some kinds of alloantigens may be induced by C-type virus indicates an important fundamental clue to the alteration of the establ concept of some alloantigens.

C. GSA may be classified into several different groups. Some of them may be derived from GMuLV-associated cell surface antigens and others from the virus itself. This study will provide pure antigens which will tremendously help us to develop methods of diagnosing malignancy and producing specific, cellular as well as humoral antibodies. Especially, the analysis of viral GSA may suggest some unknown mechanisms of cell-to-cell virus infection.

D., E. and F. Immunological studies on the behavior of malignant cells will suggest methods of treating tumor patients. In particular, the absence of absolute immunological tolerance to oncorna virus-associated antigens raises the question of whether it is possible to break relative immunological tolerance to both viruses and malignant cells. In combination with studies on activation of viral genome producing antigens and antigenic modulation of G cell surface antigen(s), this study may open the way to the establishment of methods of prophylaxis and treatment

Proposed Course:

The goals of these projects for the next fiscal year are:

A. To analyze the spatial relationship among different surface antigens of virus-induced tumor cells and other cells.

B. To purify different specific antigens for the preparation of mono-specific antiserum.

C. To search for a cell-mediated immune response to different specific antigens and their immunological and biological behaviors.

D., E. and F. In order to break down the relative immunological toleranc we have to further investigate naturally occurring antibody to oncogenic virus-associated antigens. For this, further investigation of G antigeni modulation and antigen forming activation will be very significant.

Honors and Awards:

Presented a paper entitled "An analysis of antigens on the surface of murine leukemia viruses and cells" at the Vth International Symposium for Comparative Leukemia Research, in Padova, Italy, September 1971.

Presented a paper entitled "Analysis of murine C-type virus-related antigens by immuno-electron microscopy", at the symposium on "Antibodies: Molecular and Cellular Aspects" of the New York Society of Electron Microscopists, in New York, New York, March 1972.

Publications:

Aoki, T., Geering, G., Beth, E., and Old, L.J.: Suppression of antigens in Burkitt's lymphoma and human melanoma cells grown in selected human sera. In Nakahar, W., Nishioka, K., Hirayama, T. and Ito, Y. (Eds.): Recent Advances in Human Tumor Virology and Immunology. Tokyo, Japan, University of Tokyo Press, 1971, pp. 425-429.

Aoki, T. and Hashimoto, Y.: Human tumor immunology. I. Chugai-Iyaku. 24: 164-174, 1971.

Aoki, T. and Hashimoto, Y.: Human tumor immunology. II. Chugai-Iyaku. 24: 223-228, 1971.

Aoki, T. and Hashimoto, Y.: Human tumor immunology. III. Chugai-Iyaku. 24: 268-276, 1971.

Aoki, T.: Surface antigens of murine leukemia cells and murine leukemia viruses. Transplant. Proc. III: 1195-1198, 1971.

Aoki, T., Wood, H.A., Old, L.J., Boyse, E.A., de Harven, E., Lardis, M.P., and Stackpole, C.W.: Another visual marker of antibody for electron microscopy. Virology 45: 858-862, 1971.

Oldstone, M.B.A., Aoki, T., and Dixon, F.J.: Activation of spontaneous murine leukemia virus-related antigen by lymphocytic choriomeningitis virus. Science 174: 843-845, 1971.

Dixon, F.J., Tonietti, G., Oldstone, M.B.A., Aoki, T., and McConahey, P.J.: The effect of chronic viral infections on NZ mice. In Miescher, P.A. (Ed.): Immunopathology, VIth International Symposium. New York, Grune & Stratton, 1972, pp. 377-384.

Oldstone, M.B.A., Aoki, T., and Dixon, F.J.: The antibody response of mice to murine leukemia virus in spontaneous infection: Absence of classical immunologic tolerance. Proc. Nat. Acad. Sci. U.S.A. 69: 134-138, 1972.

Aoki, T. and Takahashi, T.: Viral and cellular surface-antigens of murine leukemias and myelomas: Serological analysis by immuno-electron-microscopy. J. Exper. Med. 135: 443-457, 1972.

Aoki, T.: An analysis of antigens on the surface of murine leukemia viruses and cells. In Chieco-Banchi, L. and Dutcher, R.M. (Eds.): Proceedings of the Vth International Symposium for Comparative Leukemia Research. In press.

Nowinski, R.C., Fleissner, E., Sarkar, N.H., and Aoki, T.: Chromatographic separation and antigenic analysis of protein of the oncornaviruses. II. Mammalian leukemia-sarcoma virus. J. Virol. 9: 359-366, 1972.

Serial No. NCI-4845
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: A. Studies on the effect of methoxyindoles on the growth of selected murine and rat tumors

B. Studies on the inter-relationships between Plasmodium bergie infestation and murine leukemia and sarcoma viruses.

Previous Serial Number: None

Principal Investigator: Dr. Richard S. Buswell

Other Investigators: Dr. Adi F. Gazdar

Cooperating Units: Inside NIH
Dr. Robert Herman, LPD, NIAID

Outside NIH
Flow Laboratories, Rockville, Maryland

Man Years:

Total:	1.7
Professional:	1.0
Other:	0.7

Project Description

Objectives:

A. To assess the effect of parenterally administered methoxy-indoles on the growth rate of melanomas and breast carcinomas.

B. To assess the effect of concomitant malaria infestation on the growth of MSV and MuLV (Abelson) induced tumors in BALB/c mice. To assess the effect of MSV or MuLV on the course of Plasmodium bergei induced malaria in BALB/c mice.

Serial No. NCI-4845

Methods Employed:

A. Melatonin, a methoxyindole, was administered subcutaneously to the laboratory animal. Tumor transplantation followed variable periods of pretreatment with the compound. Animals were sacrificed at serial intervals and experimental tumor weights compared to controls.

B. MuLV or MSV was inoculated in mice prior to their infestation with malaria. The course of tumor growth and malaria were then monitored.

Major Findings:

A. To date only one methoxy indole has been evaluated: malatonin (5 methoxy-N-acetyl tryptamine). The compound has no significant effect on two murine melanomas tested, but has consistently enhanced the growth of a transplanted breast carcinoma in Sprague Dawley rats.

B. It appears that concomitant malarial infestation does not significantly enhance the time of appearance, growth rate or regression rate (in the case of MSV) of MSV or MuLV (Abelson) induced tumors. It appears, however, that these two viruses do affect the severity and course of P. bergei malaria (17X strain), as reflected in prolonged parasitemia, heavy parasite load, severe anemia, and shortened survival time in the experimental animals.

Significance to Biomedical Research and the Program of the Institute:

A. Several data suggest that pinealectomy enhances the growth of tumors. Since the methoxy indoles are produced almost exclusively in the pineal gland, it is relevant to investigate their effect on tumor growth.

B. The occurrence of Burkitt's lymphoma predominantly in malarial infested regions of the world, leads to speculation about malaria enhancing viral oncogenesis and tumor growth.

Proposed Course:

A. The effects of methoxyindoles other than melatonin on tumor growth will be tested.

B. The inter-relationships between malaria, oncogenic viruses and the immune system will be studied.

Honors and Awards:

None.

Publications:

None.

Serial No. NCI-4846
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: A. Biochemical studies of a human breast carcinoma line

B. Biochemical studies on human milk

C. Naturally occurring inhibitors of murine viral reverse transcriptase

D. Development of a complement dependent assay of increased sensitivity

Previous Serial Number: None

Principal Investigator: Dr. Brenda I. Gerwin

Other Investigators: Dr. Tadao Aoki

Cooperating Units: Inside NIH
Dr. Robert Bassin, VLLB, NCI
Dr. Daniel Haapala, VLLB, NCI
Dr. Vincent W. Hollis, VLLB, NCI
Dr. Ernest Plata, VLLB, NCI
Dr. Paul Ebert, VBB, NCI
Dr. Harish Chopra, VBB, NCI
Dr. Michael Frank, LCI, NIAID
Dr. Julie Milstien, LPB, NIAMD

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

A. To search an available breast carcinoma cell line for evidence of enzyme activity and high molecular RNA characteristic of known tumor viruses.

B. To determine whether enzyme activities reported in human milk are specific for a virus and/or related to a viral etiology for human breast carcinoma.

C. To check serum of untreated mice for specific inhibitors of viral reverse transcriptase to determine whether an antibody to this viral enzyme might occur in animals which are not specifically injected with virus or virus containing materials.

D. To use the enzymatic activation of the first component of complement by antigen-antibody complexes as a rapid, spectrophotometric assay for various antigens.

Methods Employed:

A. Rate zonal centrifugation monitored by incorporation of radioactive RNA precursors and reverse transcriptase assays was used to examine both cells and supernatant medium for the presence of virus-like particles.

B. Milk samples were screened by enzymatic assay for reverse transcriptase activity. Column chromatography was then used to determine whether the activity was attributable to viral or cellular enzymes.

C. Inhibition of the enzymatic activity of chromatographically purified Rauscher leukemia virus reverse transcriptase was used as a screening device for the presence or absence of anti-polymerase activity. Selected sera were tested by immune-electron microscopy for antibodies to viral envelope and/or cell surface antigens.

D. The first component of human complement was partially purified by specific precipitation and density gradient centrifugation. The protein was assayed spectrophotometrically for its esterolytic activity in the presence and absence of immune complexes.

Major Findings:

A. The established human breast carcinoma cell line in tissue culture is not producing a completed virion. Some evidence indicates that some precursors of complete virions may be present.

B. Twenty-five percent of the general population screened have an enzyme activity in their milk which might be considered viral. Further purification of milk samples by column chromatography shows no evidence for an enzyme which chromatographically behaves like a viral enzyme. When Rauscher leukemia virus is added to human milk, the viral enzyme chromatographs normally. Thus further purification indicates that the activity found on crude enzymatic assays is not viral.

C. A modified reverse transcriptase assay was developed to reduce the background inhibition shown by all sera. Using this assay, normal sera from untreated mice of different strains and rat hyperimmune serum of known reactivity toward cell surface or viral envelope antigens were examined. Polymerase inhibition to some extent correlated with reactivity against viral envelope antigens and could be selectively removed by absorption with virus-infected tumor cells. When sera from untreated animals were tested the polymerase inhibitor was found in BALB/c, Ha/ICR, C58, and C3H/He mice, but not in AKR or C57BL/6 animals. The prevalence of the inhibitor was particularly high in C58 and C3H/He mice.

D. The esterase activity of human C_1 fraction can be specifically enhanced by antigen-antibody complexes. This activation should be useful as a probe for immune complex formation in some immune systems.

Significance to Biomedical Research and the Program of the Institute:

A. Many provocative recent reports indicate that human breast carcinoma may show evidence of a viral etiology. The availability of a variety of tissue culture cell lines allows a biochemical investigation of large quantities of material. We hope to determine whether these cells contain any unique viral specific components.

B. Our findings in human milk suggest that the polymerase enzymes present there are not related to the enzymes of the known tumor viruses.

C. The presence of polymerase inhibitors in the sera of some but not all mouse strains provides a model system for the examination of problems relevant to the sensitivity of an individual to tumor viruses and detection of natural exposure to tumor virus.

D. The development of a more sensitive and less complicated probe for antigen-antibody reactions will be very useful in the search for these reactions in appropriate immune systems of human origin.

Proposed Course:

A. It will be determined whether the particles in the breast tumor cell line are viral precursors and whether they are unique to that cell line.

B. We will isolate polymerases from human cells to see whether, when mixed with milk and then purified, they have the characteristics of polymerases which have been isolated from human milk.

C. We will determine whether the natural polymerase inhibitors are antibodies and whether they inhibit only viral polymerases.

D. The C_1 activation assay will be made quantitative and its sensiti will be examined in known soluble and cellular systems. For this purpo we must overcome the anticomplementary effect and endogeneous C_1 of test materials.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4847
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Morphologic studies on virus induced tumors

Previous Serial Number: None

Principal Investigator: Dr. Yoji Ikawa

Other Investigators: Dr. Adi F. Gazdar
Dr. Stuart A. Aaronson
Dr. Tadao Aoki

Cooperating Units: Inside NIH
Dr. Harish C. Chopra, VBB, NCI
Dr. Paul S. Ebert, VBB, NCI
Dr. Sachio Nabeshima, LNNS, NINDS

Outside NIH
Dr. T.N. Fredrickson, Univ. of Conn., Storrs, Conn.
Dr. William C. Moloney, Peter Bent Brigham Hospital
Boston, Massachusetts
Dr. M. Furusawa, Osaka City University, Osaka, Japan
Dr. M. Hatanaka, Flow Labs, Inc., Rockville, Md.

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

A. To study the morphology, and degree of hematological differentiation of Rauscher virus (RLV) induced tumor cell culture lines.

B. To study the biological behavior and morphology of murine sarcoma virus (MSV) "non-producer" cell culture lines.

Methods Employed:

Routine tissue culture methods including methods used to obtain single cell clones. Muramidase assays are performed by an agar gel immunoplate method. Phagocytic activity is studied by uptake of yeast organisms and India ink granules. Electron microscopic visualization of peroxidase is carried out. Spleen coloney formation is done by inoculation of cloned cells into lethaly irradiated mice. Erythrocytic maturation of clonal Friend leukemia lines after DMSO treatment is studied by a fluorescein-labelled erythrocyte membrane-specific antibody. Switching on of heme synthetase are studied after DMSO treatment. Viral RNA-DNA transcriptase label is checked after DMSO treatment.

Major Findings:

Clonal cell lines of Rauscher virus-induced monocyelocytic lines (RFD line) were established, which were positive for muramidase and peroxidase production, and which exhibited phagocytic activity. Morphological erythroblasts were observed in the coloney cells. When transplanted into syngenic mice, "starry sky patterns" were presented. Culture fluids contained approximately 5×10^2 ffu/ml of MuLV, and induced erythroleukemia when inoculated into newborn BALB/c mice. Annualate lamellae of the FRD line were peroxidase-positive.

Erythrocytic membrane-specific antigens were present on the cells of primary splenic lesions of Friend leukemia, but not on the cells of Friend leukemia lines. However, the antigens were detectable on Friend leukemia lines after treatment with 2% DMSO. The MuLV titer of culture fluids of the same lines were increased more than 10^2 FFU after DMSO treatment. Intracysternal particles, and peculiar particles with duplicate nucleoids were observed in the cells.

Kirsten MSV-transformed BALB/3T3 non-producer clones were morphologicallv epithelial when transplanted into syngenic mice. The non-producer cells regressed in the syngenic hosts when they were superinfected with naturally occurring virus (B-tropic, Gross type) or with experimentally obtained viruses (Moloney MLV, FLV, or RLV). KiMSV non-producer-produced transplants were negative for murine gs antigens even after the ten *in vivo* passages. The *in vitro* morphologv of viral transformants does not always correlate with the in vivo epithelial features.

Significance to Biomedical Research and the Program of the Institute:

A detailed study of the ability of oncogenic viruses to induce tumors of different origin histology and degree of differentiation will help to understand the large variety of tumors arising in humans.

Proposed Course:

Possibility of myelomonocytoid lines coverting to erythrocytic series will be studied. Possible methods of treatment of leukemia by inducing differentiation of leukemic cells will be studied.

Honors and Awards:

Dr. Ikawa is an awardee of Fogarty International Fellowship (Jan 4, 1971 to Jan 3, 1973).

He presented a paper entitled "Erythrocyte Membrane-specific Antigens on Friend Virus-induced Leukemia Cells" at the Vth International Symposium on Comparative Leukemia Research at Padova, Italy, September 13-17, 1971.

Dr. Ikawa presented a seminar entitled "Pathogenesis of Friend Virus-induced Leukemia" at Department of Animal Disease, University of Connecticut, Storrs, Connecticut on October 22, 1971.

Dr. Ikawa will present a paper entitled "Rauscher virus-induced myelomonocytic leukemia culture lines" at the annual American Association of Cancer Research meeting, Boston, Massachusetts, May 1972.

Publications:

Ikawa, Y., Furusawa, M., and Sugano, H.: Erythrocyte-membrane-specific antigens on Friend virus-induced leukemia cells, Bibl. Haemat. In press

Sugano, H., Furusawa, M., Kawaguchi, T., and Ikawa, Y.: Substances to enhance erythrocytic maturation of Friend leukemia lines, Bibl. Haemat. In press

Ikawa, Y., Sugano, H., and Furusawa, M.: Pathogenesis of Friend virus-induced leukemia in mice, GANN Monograph 12 In press

Serial No. NCI-4848
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: A. Studies with murine sarcoma virus (MSV)-transformed mouse 3T3 cells which are negative for leukemia virus

B. Isolation and characterization of a new human breast tumor cell line.

Previous Serial Number: NCI-4823

Principal Investigator: Dr. Robert H. Bassin

Other Investigators: Dr. Stuart Aaronson
Dr. Peter J. Fischinger
Dr. Brenda Gerwin
Dr. Daniel Haapala
Dr. Shigeko Nomura
Dr. Leo A. Phillips
Dr. Paul T. Peebles
Dr. Ernest J. Plata

Cooperating Units: Inside NIH
Staff, VLLB, NCI
Dr. Carl F. T. Mattern, LVD, NIAID

Outside NIH
None

Man Years:

Total:	2.3
Professional:	1.0
Others:	1.3

Project Description

Objectives:

A. To characterize clonal isolates of 3T3 cells transformed by murine sarcoma virus in the absence of replicating leukemia virus.

B. To isolate and characterize a continuous cell line of human breast tumor cells.

Methods Employed:

A. Standard cell culture procedures were employed including the cloning of cells in microplates, transformation, and focus assays in a variety of cell types both in monolayers and in agar suspension cultures. A new cell culture assay procedure for murine leukemia virus, described in last year's annual report, was routinely used. Standard techniques for virus detection by incorporation of radioactive precursors as well as by electron microscopic examination were also employed.

B. Standard cell culture procedures were employed including the cloning of cells in microplates, transformation, and focus assays in a variety of cell types both in monolayers and in agar suspension cultures. Standard techniques for virus detection by incorporation of radioactive precursors as well as by electron microscopic examination were also employed.

Major Findings:

A. Virological properties of S+L- cells were examined. Quantitative studies on the rescue of MSV from S+L- cells following superinfection with MuLV showed that both progeny MuLV and rescued MSV are released at approximately the same rate. There is no early burst of MSV synthesis detectable prior to MuLV production. That portion of the MuLV genome necessary for MSV rescue was estimated by measuring the UV inactivation rate for MuLV rescue activity as compared to the inactivation rate for MuLV replicating activity. The two rates were found to be identical. Neutralization experiments showed that MSV rescued for S+L- cells acquires the type specific envelope antigen of the MuLV strain used for rescue.

The above results indicate that some late function in the MuLV replicative cycle such as synthesis of envelope antigen is necessary for the rescue of defective MSV from S+L- cells. Previous data have indicated that MuLV may provide additional helper functions in the system.

B. A continuous cell line, HBT-3, was established in culture from a human breast adenocarcinoma. The cells are epithelioid in appearance, grow rapidly, and form multilayered colonies. HBT-3 cells have a cloning efficiency of approximately 70% and an abnormal karyotype (model chromosome number = 66).

Significance to Biomedical Research and the Program of the Institute:

A. An understanding of the "defectiveness" of viruses such as MSV would be of obvious value to efforts attempting to detect viral genomes in tumor cells of unknown etiology when no infectious virus is evident. The interactions of defective sarcoma viruses with leukemia helper viruses may also be of value in studies on the mechanism of cell transformation by oncogenic RNA viruses.

B. Recent studies with human breast tumor material and with human milk samples have indicated the presence of 65-70S RNA, reverse transcriptase, and virus-like particles in patients with a high risk of breast carcinoma.

Since there are very few continuous human breast tumor lines in existence, the HBT-3 line should be useful in studies on the relationship of RNA tumor viruses to human breast cancer.

Proposed Course:

A. Efforts to study the nature of the defectiveness of MSV as well as the role of leukemia helper virus will be continued.

B. Human breast tumor line HBT-3 will be used in attempts to detect and characterize reverse transcriptase, high molecular weight RNA and virus particles which are associated with human breast tumors in vivo.

Honors and Awards:

Dr. Bassin presented a paper entitled "Properties of 3T3 cells transformed by murine sarcoma virus in the absence of replicating murine leukemia helper virus" at the Vth International Symposium on Comparative Leukemia Research, Padua, Italy, September 1971.

Publications:

Bassin, R. H., Phillips, L. A., Kramer, M. J., Haapala, D. K., Peebles, P. T., Nomura, S., and Fischinger, P. J.: Transformation of mouse 3T3 cells by murine sarcoma virus: Release of virus-like particles in the absence of replicating murine leukemia helper virus. Proc. Nat. Acad. Sci. U.S.A. 68: 1520-1524, 1971.

Bassin, R. H., Phillips, L. A., Kramer, M. J., Haapala, D. K., Peebles, P. T., Nomura, S., and Fischinger, P. J.: Properties of 3T3 cells transformed by sarcoma virus in the absence of replicating murine leukemia helper virus. In Chieco-Banchi, L. and Dutcher, R. M. (Eds.): Proc. Vth International Symposium on Comparative Leukemia Research. In press.

Gazdar, A. F., Russell, E., and Bassin, R. H.: In vivo enhancement of a murine sarcoma virus by diethylaminoethyl-dextran. Proc. Soc. Exp. Biol. Med. 137: 310-314, 1971.

Turner, W., Ebert, P. S., Bassin, R. H., Spahn, G., and Chirigos, M. A.: Potentiation of murine sarcoma virus (Harvey) (Moloney) oncogenicitv in Lactic Dehydrogenase-elevating virus-infected mice. Proc. Soc. Exp. Biol. Med. 136: 1314-1318, 1971.

Gazdar, A. F., Sarma, P. S., and Bassin, R. H.: Properties of a murine sarcoma virus isolated from a tumor arising in a NZW/NZB F_1 hybrid mouse. II. Physical and biological characteristics. Int. J. Cancer 9: 234-241, 1972.

Peebles, P. T., Bassin, R. H., Haapala, D. K., Phillips, L. A., Nomura, S., and Fischinger, P. J.: Rescue of murine sarcoma virus from a sarcoma positive leukemia negative cell line: Requirement for replicating leukemia virus. J. Virol. 8: 690-694, 1971.

Nomura, S., Bassin, R. H., Turner, W., Haapala, D. K., and Fischinger, P. J.: Ultraviolet inactivation of Moloney leukemia virus: Relative target size required for virus replication and rescue of "defective" murine sarcoma virus. J. Gen. Virol. In press.

Nomura, S., Bassin, R. H., and Fischinger, P. J.: Replication of radiation induced murine leukemia virus (RadL^{V}) in normal and transformed mouse cells. J. Virol. In press.

Aaronson, S. A., Bassin, R. H., and Weaver, C.: Comparison of murine sarcoma viruses in nonproducer and S+L- transformed cells. J. Virol. In press.

Serial No. NCI-4849
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Transformation of human embryonic kidney cells

Previous Serial Number: NCI-4888, 4889, 4891, 4897, 4843

Principal Investigator: Dr. John M. Easton

Other Investigators: Dr. Dharam V. Ablashi

Cooperating Units: Inside NIH
Dr. H. Chopra, VBB, NCI

Outside NIH
Dr. David Fabrizio
Bionetics, Kensington, Md.

Man Years:

Total:	1.5
Professional:	1.0
Other:	0.5

Project Description

Objectives:

To study transformation of human embryonic kidney (HEK) cells in cell culture after infection with Rauscher leukemia virus and subsequent incubation with varying concentrations of 3-methylcholanthrene (3-MC). The role of occult viruses in the original HEK preparation is also being studied.

Methods Employed:

Kidney cell cultures from a 1000 gram male fetus (27 weeks) were infected with each of 2 strains of Rauscher leukemia virus previously found to replicate in human cells. Subsequent to this, the cell cultures were incubated with varying concentrations of 3-MC and then with maintenance medium. The cell cultures were followed microscopically

for evidence of change. Special techniques are being used for viral enhancement and detection, such as complement fixation, negative staining, karyotypic analysis, and treatment with Iud.

Major Findings:

Transformed cells arose in all cultures - those which were untreated, those receiving either virus or 3-MC, and those receiving both virus and 3-MC. Evidence for transformation was an increased cellular replication rate, growth out of the monolayer with jackstraw appearance, cells with more than one nucleus, and karyotypic abnormalities. Transformation appears to have occurred both spontaneously and repetitively.

Significance to Biomedical Research and the Program of the Institute:

The study of transformed human cells is of interest in that it may reveal possible human viruses which are oncogenic. Investigation of a possible virus which leads to the transformation of HEK cells may provide clues to viruses which cause tumor in vivo.

Proposed Course:

Spontaneous transformation of HEK cells is a relatively rare event. Studies are being and will be carried out to determine the relationship of the transformation to a possible occult virus in the original preparation, and the relationship of virus and treatment with methylcholenthrene. Iud is being used in an attempt to augment viral replication.

Honors and Awards:

None

Publications:

Stevens, D. A., Easton, J. M., Levine, P. H., Waggoner, D. E., Manaker, R. A., and Schidlovsky, G.: Antilymphocyte serum and lymphoid cell-virus carrier culture: cell kinetics, morphology, EB virus replication, interferon. Cell. Immun. In press.

Armstrong, G. R., Ablashi, D. V., Easton, J. M., and Adamson, R. H.: Suppression of the cytopathic effects of Herpesvirus saimiri by cytosine arabinoside in primary owl monkey cells. Proc. Amer. Soc. Micro. In press.

Serial No. NCI-4850
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: A. Replication of radiation-induced murine leukemia virus (RadLV) in normal and transformed mouse cells.

B. Determination of relative sizes of the murine leukemia virus (MuLV) genome required for virus replication and for rescue of defective murine sarcoma virus (MSV), and the MSV genome required for cell transformation.

C. Isolation and characterization of variant cell lines from mouse cells transformed by MSV.

Previous Serial Number: NCI-4839

Principal Investigator: Dr. Shigeko Nomura

Other Investigators: Dr. Peter J. Fischinger
Dr. Robert H. Bassin
Dr. Paul T. Peebles

Cooperating Units: Inside NIH
Staff, VLLB, NCI
Dr. Carl F. T. Mattern, LVD, NIAID
Mr. Horace C. Turner, VC, NCI

Outside NIH
None

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

A. To study the replication of RadLV in mouse cell cultures and the defectiveness of RadLV.

B. To compare the relative sizes of the MuLV genome required for virus replication with that necessary for rescue of defective MSV from transformed 3T3 cells (S+L- cell line), and with the MSV genome required for cell transformation.

C. To isolate variant cell lines with a reversion from properties of the S+L- cells to properties resembling untransformed parental cells.

Methods Employed:

A. Routine tissue culture methods and standard virological techniques for the quantitation of cell transformation and focus formation by mouse sarcoma and leukemia viruses were used.

B. Routine tissue culture methods and standard virological techniques for the quantitation of cell transformation and focus formation by mouse sarcoma and leukemia viruses were used. Relative genome sizes were determined following stepwise inactivation with ultraviolet light (UV). 5-iododeoxy-uridine (IUDR) treatment was carried out by exposing cells to 100 µg/ml of of IUDR for 24 hours.

C. Routine tissue culture methods and standard virological techniques for quantitation of cell transformation and focus formation by mouse sarcoma and leukemia viruses were used. Reverse transcriptase activity was determined by [^{3}H-methyl] thymidine triphosphate incorporation into acid precipitable material.

Major Findings:

A. Autonomous RadLV replication could be detected in mouse 3T3 cells by the development of interference with MSV, the appearance of covert helper activity for defective MSV, and by the induction of CPE type foci in S+L- cells. A chronic infection of either 3T3 or S+L- cells with RadLV could be established. Both RadLV infectivity and helper activity were demonstrated in the same peak at a buoyant density of 1.16 g/cm^3. Additionally a soluble inhibitor of MSV focus formation was found which could be separated from infectious RadLV. Examination of cell clones derived from chronically infected 3T3 cells showed that essentially every cell was infected and produced both infectious RadLV and low levels of inhibitor. Quantitative comparisons of autonomously replicating RadLV in normal 3T3 and S+L- cells suggested that RadLV may consist of several populations of virus of varying replicative potential. Apparently 99% of RadLV can be assayed only as helper units in normal cells or as replicative units in S+L- cells.

B. The ability of MuLV to rescue MSV from S+L- cells and its ability to replicate were equally sensitive to UV irradiation. The entire genome of MuLV appeared to be necessary for the rescue of defective MSV. The MSV focus forming activity was 1.4 times more resistant to UV irradiation than was MuLV replicating activity.

C. Variant cell lines which demonstrated reversion to some properties of untransformed cells were isolated from S+L- cell lines. Such variant cells were epithelioid, contact inhibited, and their cloning efficiency in soft agar and saturation density were similar to normal 3T3 cells. They contained some MuLV group specific antigens, but demonstrated no virus production, and no reverse transcriptase activity. MSV could no longer be rescued from these variant cells. An enhancement of sensitivity to MSV and MuLV infection was observed in variant cell cultures. Variant clones spontaneously gave rise to morphological re-transformation during cultivation in vitro. Reverse transcriptase activity and virus particles which were not infectious to the original host cells, were induced in one tertiary variant clone and one spontaneously re-transformed clone after treatment with IUDR. Chromosome studies of these variants indicated that increase in chromosome number accompanied reversion of properties of transformation, and decrease in chromosome number accompanied re-transformation.

Significance to Biomedical Research and the Program of the Institute:

A. An understanding of the defectiveness of RadLV and the influence of this virus on host control mechanisms may suggest a more efficient approach to the in vitro replication of other oncorna viruses.

B. A genetic analysis of different functions of oncogenic viruses may provide the information on the nature and mechanism of viral oncogenesis.

C. Studies of the behavior of the viral genome in cells transformed by virus are not only of general biological interest but may also provide new approaches to the search for etiologically significant latent viruses in tumors, and for possible therapeutic measures.

Proposed Course:

A. Further studies on the nature of the defectiveness of RadLV and inhibitor(s) produced in chronically infected 3T3 cells.

B. Further studies on the nature of the defectiveness of MSV by stepwise inactivation with physical and chemical agents.

C. Further studies on the optimum conditions for selection of the variant cells, the state of integration of the viral genome, chemical or physical agents which induce reversion of transformed cells, the effect of low or high temperatures on transformed and reversion states, and a possible functional change in the cell membrane of variant cell lines.

Honors and Awards:

None

Publications:

Nomura, S., Bassin, R.H., Turner, W., Haapala, D.K., and Fischinger, P.J.: Ultraviolet inactivation of Moloney leukaemia virus: Relative target size required for virus replication and rescue of "defective" murine sarcoma virus. J. Gen. Virol. In press.

Nomura, S., Bassin, R.H., and Fischinger, P.J.: Replication of radiation induced murine leukemia virus (RadLV) in normal and transformed mouse cells. J. Virol. In press.

Bassin, R.H., Phillips, L.A., Kramer, M.J., Haapala, D.K., Peebles, P.T., Nomura, S., and Fischinger, P.J.: Transformation of mouse 3T3 cells by murine sarcoma virus: Release of virus-like particles in the absence of replicating murine leukemia helper virus. Proc. Nat. Acad. Sci. U.S.A. 68: 1520-1524, 1971.

Peebles, P.T., Bassin, R.H., Haapala, D.K., Phillips, L.A., Nomura, S., and Fischinger, P.J.: Rescue of murine sarcoma virus from a sarcoma-positive leukemia-negative cell line: Requirement for replicating leukemia virus. J. Virol. 8: 690-694, 1971.

Bassin, R.H., Phillips, L.A., Kramer, M.J., Haapala, D.K., Peebles, P.T., Nomura, S., and Fischinger, P.J.: Properties of 3T3 cells transformed by murine sarcoma virus in the absence of replicating murine leukemia helper virus. In Chieco-Banchi, L. and Dutcher, R.M. (Eds.): Proceedings Vth International Symposium on Comparative Leukemia Research. In press.

Serial No. NCI-4851
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on the soluble complement fixing antigen associated with the Epstein-Barr virus genome.

Previous Serial Number: None

Principal Investigator: Dr. Stephen C. Marker

Other Investigators: Dr. David M. Livingston
Dr. George J. Todaro

Cooperating Units: Inside NIH
Dr. Wade P. Parks, VCB, NCI

Outside NIH
Meloy Laboratories, Inc.
Springfield, Virginia

Man Years:

Total:	1.0
Professional:	0.9
Other:	0.1

Project Description

Objectives:

1. To isolate the soluble complement fixing antigen associated with the Epstein-Barr virus genome.

2. To develop a sensitive specific assay for the antigen.

3. To investigate the distribution of the antigen in various tissues and tumors.

Methods Employed:

Standard biochemical protein fractionation procedures: DEAE cellulose and hydroxylapatite chromatography, isoelectric focusing, and affinity chromatography. For detection of the antigen: complement fixation and radioimmunoassay.

Major Findings:

The antigen is heat labile, elutes from DEAE with 0.25M NaCl, elutes from hydroxylapatite with 0.1M KPO_4, has its isoelectric point at pH 4.8, and has a molecular weight on SDS polyacrylamide gels of approximately 80,000.

Significance to Biomedical Research and the Program of the Institute:

This antigen appears to be present in cells that contain the Epstein-Barr virus genome, in the absence of whole virus production. As such, it may lead to a better understanding of the general problem of latent Herpes virus infections, such as recurrent Dendritic ulcer, Herpes Zoster, and Herpes Labalis. My study of this antigen will help determine the role of the Epstein-Barr virus in infectious mononucleosis, Burkitt's lymphoma, and other human tumors.

Proposed Course:

The soluble antigen will be purified to homogeneity, a radioimmunoassay developed, and the distribution of the antigen determined in a variety of cell lines, human tissues, and tumors.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4852
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The search for a murine leukemia virus repressor and in vitro translation of the murine sarcoma virus genome

Previous Serial Number: None

Principal Investigator: Dr. Garth Anderson

Other Investigators: Dr. Stuart A. Aaronson
Dr. John Stephenson
Dr. George J. Todaro

Cooperating Units: Outside NIH
Meloy Laboratories, Inc.
Springfield, Virginia

Man Years:

Total:	0.9
Professional:	0.8
Other:	0.1

Project Description

Objectives:

1. To synthesize in vivo DNA corresponding to murine leukemia virus RNA.

2. To define the conditions of viral-coded DNA synthesis.

3. To utilize double stranded viral coded DNA as a probe for a repressor of the latent leukemia virus present in normal mouse cells.

4. To translate in vitro 70S RNA from murine sarcoma virus and to define the products thereof.

Methods Employed:

Biochemical techniques, such as DNA-RNA hybridization, are being used to characterize viral DNA. A nitrocellulose filter binding assay is being

developed to search for a viral DNA-binding repressor protein. Krebs Ascites cells grown in mice are being used to synthesize viral proteins in vitro, with the eventual goal of identifying such proteins as viral by radioimmunoassay procedures.

Major Findings:

Systems to explore and define in vivo DNA synthesis from the viral genome are in development. 70S RNA in quantity from a sarcoma/leukemia mix has been prepared and the translation system for in vitro protein synthesis is being developed.

Significance to Biomedical Research and the Program of the Institute:

These studies are aimed at the elucidation of the molecular mechanism controlling the expression of endogenous leukemia viruses, and exploring the events surrounding DNA synthesis of the viral RNA genome. The preparation of labelled protein coded for by the murine sarcoma virus is of potential use in determining the molecular mechanism of transformation.

Proposed Course:

To follow leads in the program developed during the past year and to extend them with a goal of providing an understanding of human cancer.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4853
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Detection of oncogenic virus specific information by molecular hybridization in mammalian cells.

Previous Serial Number: None

Principal Investigator: Dr. Raoul Benveniste

Other Investigators: Dr. Edward M. Scolnick
Dr. George J. Todaro

Cooperating Units: Outside NIH
Meloy Laboratories, Inc.,
Springfield, Virginia

Man Years:

Total:	1.0
Professional:	0.9
Other:	0.1

Project Description

Objectives:

1. To examine various murine and human transformed and "normal" cells for the presence of C-type viral information.

2. To determine the relatedness of C-type viruses isolated from various species.

Methods Employed:

Nucleic acid hybridization is the principal method that is being used. A rapid and sensitive assay has been developed to detect RNA-DNA and DNA-DNA hybrids by the use of various nucleases that will degrade only single stranded nucleic acids, leaving hybrid structures intact.

Major Findings:

A nuclease from Aspergillus oryzae and one from mung bean sprouts have bee

partially purified by standard chromatographic techniques. Assay conditions have been developed that allow the use of these enzymes for the detection of RNA-DNA hybrids. These assays are more convenient and more sensitive than the more common techniques of hydroxylapatite chromatography or $CsSO_4$ equilibrium centrifugation in the detection of double stranded nucleic acids.

Using this assay, it has been possible to detect hybridization between product DNA made in vitro from Kirsten sarcoma and leukemia virus by using reverse transcriptase and total cellular RNA extracted from various cells. The RNA from every mouse cell line tested to date, which includes such lines as Balb/3T3, and NIH/Swiss mouse embryo fibroblasts (NIH/3T3) has been found to hybridize to product DNA made from Kirsten sarcoma and leukemia virus. Cell lines transformed by SV40 and Kirsten or Moloney sarcoma virus show increased levels of hybridization.

Kirsten sarcoma and leukemia virus product DNA has also been shown to hybridize to Woolly monkey and Gibbon ape infected human fibroblasts. Experiments are currently being performed that will reveal any direct homology between Woolly and Kirsten viruses.

Kirsten leukemia virus product DNA has been shown to hybridize with the RNA extracted from Kirsten sarcoma virus transformed nonproducer normal rat kidney cells. This data suggests that there might be some homology between Kirsten leukemia and sarcoma viruses; alternatively, it is possible that the sarcoma virus is inducing an endogenous leukemia virus that bears some homology to Kirsten leukemia virus.

Significance to Biomedical Research and the Program of the Institute:

The discovery that cross-species hybridization among C-type viruses (such as Kirsten leukemia and sarcoma viruses, Moloney sarcoma virus, and Woolly monkey and Gibbon ape viruses) exists implies that detection of C-type information in human tumors is technically feasible.

The finding of murine C-type viral information in "normal" murine cell lines also has strong implications for a viral etiology for cancer.

Proposed Course:

1. To search for C-type viral information in human tumors by hybridization between cellular RNA extracted from these tumors and DNA made from various C-type viruses.

2. To examine the homology between various C-type viruses by hybridization.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4854

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Etiology
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: A. Affinity chromatography of RNA tumor viral specific proteins

B. Studies on the mechanism of host cell control of transforming viral gene expression

Previous Serial Number: None

Principal Investigator: Dr. David M. Livingston

Other Investigators: Dr. Edward M. Scolnick
Dr. George J. Todaro
Dr. Stuart A. Aaronson

Cooperating Units: Inside NIH:
Dr. Wade P. Parks, VCB, NCI

Outside NIH:
Meloy Laboratories, Inc., Springfield, Virginia

Man Years:

Total:	1.1
Professional:	1.0
Other:	0.1

Project Description

Objectives:

A. Affinity chromatography has been proven to be an effective means for the rapid isolation of highly purified proteins in biological systems. A number of enzymes have been isolated using solid phase adsorbents coupled to ligands or antibody molecules having high affinity for the specific proteins desired. In this study, we have sought to establish methods which will allow one-step isolation of both the reverse transcriptase and the "group specific protein" of the RNA tumor viruses from crude cellular extracts. The major objective is to isolate such proteins from human cells thereby denoting the existence of an RNA tumor virus genome within the complement of cellular genes.

B. The goal of these studies is to determine which genes of a normal host cell are involved in the development of the transformed state following tumor

virus infection, and if present, how their products function.

Methods Employed:

A. The basic method is chromatography of crude cell extracts on solid phas materials coupled to specific anti-polymerase or anti-gs protein antibodies Other standard tissue culture, immunologic, and biochemical methods are employed as adjuncts in these studies.

B. Cell biological, tissue culture, virological, and genetic methods.

Major Findings:

A. Anti-polymerase immunoadsorbents: Specific anti-murine viral polymera and anti-avian viral polymerase antibodies (the purified IgG fractions) hav been coupled to Sepharose using the standard CNBr procedure. The coupled immunoadsorbents, poured into columns, are capable of binding only the respective viral reverse transcriptases to which the antibodies bind in solution. As an example, the anti-murine column binds the enzymes from mouse, cat and Gibbon ape C-type virus but not that of avian C-type virus. In solution, there is definitive evidence for inhibition by this antibody of the reverse transcriptases of mammalian but not avian origin. The immunoadsorbent has little or no affinity for normal host cell DNA polymer- ases. Enzyme, once bound to the column, can be recovered in active form b elution with NH_4OH. Most importantly, in both mammalian and avian systems the viral reverse transcriptase from extracts of cells transformed by homologous RNA tumor viruses and actively producing virus can be isolated in highly purified form in one step. No such enzyme either binds to or can be eluted from these columns when extracts or normal cells are chromatographed. Enzyme which is eluted from the columns has the same template requirements as conventionally purified protein and its activity, like the latter, is also inhibitable by addition of antibody in solution. To date, "viral" reverse transcriptase has been isolated from untransformed IudR induced Balb/c 3T3 cells and from the S^+L^- type of transformed nonproducer cell, but has not been detected even after scaling up the amounts of protein chromatographed and the size of the aliquots assayed, i either uninduced normal or NP types of nonproducer cells.

Large scale screening of human cell extracts is planned and will be undertaken when a high titered antibody directed against the primate C-type viral reverse transcriptase is available.

Anti-gs protein immunoadsorbents: A similar Sepharose immunoadsorbent has been prepared with anti-feline gs antibody. This serum contains antibody directed against two antigenic determinants on the gs molecular- gs_1, a species specific polypeptide sequence, and gs_3, a common antigen present in all C-type mammalian viral gs proteins. We have taken advantage of the latter feature of this serum by establishing a chromatographic system potentially capable of binding any mammalian gs protein having the gs_3 determinants.

To this end, we have recently shown that I^{125} labelled murine and feline gs antigen will bind specifically to the anti-gs_3 immunoadsorbent and not to a control column containing Sepharose coupled to non-immune rabbit IgG. The labelled protein can be isolated and concentrated from a huge volume of either buffer or crude extract. Furthermore, upon elution of the material from the column with NH_4OH and neutralization, the protein regains its antigenic properties and can be rechromatographed on the antibody column again with excellent recovery. The system is being presently prepared for studies with human tumor cell extracts in an attempt to physically isolate the gs protein where present.

B. The primary aim of the first stage of this long term project is to isolate mutant cells which, although transformed by murine sarcoma virus (MSV), are conditionally defective in their ability to remain transformed. The phenotype desired is a cell which is infected with MSV, is transformed, and therefore piles up on a dish at 32°C, but appears normal and contact inhibited at 38°C.

To date, we have succeeded in isolating several cloned mutant lines of cells which are derived from the KNRK cloned line of rat kidney fibroblasts transformed by the Kirsten strain of MSV (KiMSV). None of these cells produces virus. The mutants all appear "flat" or non-piled up at 38°C and at 32°C. Two of them have been studied in detail. In brief, these two mutants grow to saturation densities 5-10-fold lower than the parent KNRK. Both contain an endogenous MSV genome as shown by rescue experiments with leukemia (MuLV) virus and chemical induction experiments with IudR. However, these cells are relatively resistant to C-type virus infection and cannot be supertransformed by MSV. Therefore, although they contain a rescuable MSV endogenous genome, leukemia virus rescue proceeds at a much lower efficiency than in KNRK. Furthermore, independent tests of the ability to support MuLV replication in the latter two cell lines reveal it to be diminished. The cells apparently present a block to MuLV replication from other species than mouse, since rat leukemia virus also replicates at a much lower level than in the wild type cells. The defect in viral replication is apparently an early step since the efficiency of IudR induction of the MSV genome is identical in KNRK, NRK, and both mutants. This suggests that chemical induction involves replication of virus in single cells rather than release of MuLV from certain cells and recruitment by rescue of MSV from other adjacent or neighboring cells.

The implication of these results is that there is a relationship between reversion from the transformed state and C-type viral production. To more firmly establish this point, rerevertants of both mutants to the fully transformed state are presently being selected in an attempt to determine whether their ability to support MuLV replication has also increased.

At the present time, improved methods of mutant selection are being sought in an attempt to find cells which are temperature sensitive in the transformation property.

Significance to Biomedical Research and the Program of the Institute:

A. This project is directed to finding a human tumor virus.

B. This project is directed to trying to understand the mechanism of virus induced malignant transformation.

Proposed Course:

A. The goal is to identify, where present, viral reverse transcriptase in human cells. The specific approach is that described above, using an anti-primate C-type viral enzyme as the probe. Multiple human extracts will be screened when the appropriate antibody is available.

B. We shall continue to attempt selection of a temperature sensitive transformed cell using a variety of selective techniques. When such a cell is available, it will become important to identify those biochemical elements which are responsible for the phenotype shift.

Honors and Awards:

None.

Publications:

Livingston, D.M., Scolnick, E., Parks, W., and Todaro, G.J.: Affinity chromatography of RNA tumor virus reverse transcriptase on a solid phase immunoadsorbent. Proc. Nat. Acad. Sci. U.S.A. 69: 393-397, 1972.

Serial No. NCI-4855
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on the structure and function of the RNA from RNA tumor viruses and on the synthesis of DNA complements of purified mammalian messenger RNA's.

Previous Serial Number: None

Principal Investigator: Dr. Jeffrey Ross

Other Investigators: Dr. Edward Scolnick
Dr. Stuart Aaronson
Dr. George Todaro

Cooperating Units: Inside NIH
Dr. Phillip Leder, LMG, NICHD
Dr. Heim Aviv, LMG, NICHD

Outside NIH
Meloy Laboratories, Inc.
Springfield, Virginia

Man Years:

Total:	1.1
Professional:	1.0
Other:	0.1

Project Description

Objectives:

1. To purify and to analyze the structure of the 60-70S viral RNA.

2. To compare the size and subunit structure of leukemia with sarcoma viruses.

3. To synthesize DNA complements of natural mammalian mRNA's using purified viral reverse transcriptase.

Methods Employed:

Standard biochemical purification and analytical techniques.

Major Findings:

1. Techniques for the reproducible extraction of 60-70S virion RNA have been developed. The RNA of leukemia-sarcoma virions has been shown to contain an adenylate-rich region of 4-5S. The poly (A) sequences in the viral RNA amount for about 5% of the total nucleotide composition.

2. The 60-70S and subunit RNA's of leukemia and leukemia-sarcoma virus stocks appear to be indistinguishable in molecular size, as determined by SDS-polyacrylamide gel electrophoresis. However, the sedimentation constant of the 60-70S leukemia RNA in sucrose in 0.1M sodium chloride is greater than that of leukemia-sarcoma RNA.

3. High molecular weight DNA's which are complementary to rabbit and human globin mRNA's have been synthesized using purified viral reverse transcriptase.

Significance to Biomedical Research and the Program of the Institute:

A better understanding of the structure and function of viral nucleic acid is necessary to gain insight into the growth, replication, and carcinogenic properties of these viruses. DNA complementary to mRNA may be a significant tool for understanding the function of cellular mRNA's.

Proposed Course:

Follow the leads in the program developed during the past year and to extend them with a goal of proving a viral etiology of human cancer.

Honors and Awards:

None

Publications:

Ross, J., Aviv, H., Scolnick, E., and Leder, P.: In vitro synthesis of DNA complementary to purified rabbit globin mRNA. Proc. Nat. Acad. Sci. U.S.A. 69: 264-268, 1972.

Serial No. NCI-4856
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Genetic studies of induction and replication of RNA tumor viruses.

Previous Serial Number: NCI-4838

Principal Investigator: Dr. John R. Stephenson

Other Investigators: Dr. Stuart A. Aaronson
Dr. George J. Todaro
Dr. Edward M. Scolnick

Cooperating Units: Inside NIH
Dr. Tadao A. Aoki, VLLB, NCI

Outside NIH
Dr. Arthur A. Axelrad, University of Toronto, Toronto, Canada

Man Years:

Total:	1.1
Professional:	1.0
Other:	0.1

Project Description

Objectives:

1. To isolate and characterize temperature sensitive mutants of murine leukemia viruses and to use these mutants in an attempt to elucidate the mechanism of murine leukemia virus replication and to examine the functional relationships between murine leukemia and sarcoma viruses.

2. To biochemically examine the genetic relatedness between murine leukemia and sarcoma viruses.

3. To study the phenotype expression of the sarcoma virus genome in MSV-transformed "nonproducer" mouse cells.

4. To determine the genetic factors controlling induction and subsequent replication of C-type viruses from virus-free mouse embryo cell cultures.

Methods Employed:

Standard tissue culture procedures, including XC plaque assays for leukemia virus and focus formation assays for sarcoma virus, nucleic acid hybridization and *in vivo* tumor rejection.

Major Findings:

1. Micro techniques for the isolation of temperature sensitive leukemia virus mutants have been developed. By these methods, it has been possible so far to isolate 27 temperature-sensitive leukemia virus mutants from clonal stocks of Kirsten and Rauscher leukemia viruses. Each mutant transmits to new cells with efficiency comparable to that of wild-type MuLV at the permissive temperature, but is at least 4-5 logs less efficient than wild-type at forming XC plaques at the nonpermissive temperature. The mutants all have very low rates of reversion to wild-type. Preliminary characterization indicates that the temperature-sensitive blocks are post-absorption in many of the mutants. Studies will be carried out using these mutants to attempt to elucidate the mechanism of leukemia virus replication and to further examine the functional relationships between murine sarcoma and leukemia viruses.

2. The genetic relationship between Kirsten leukemia and Kirsten sarcoma virus has been examined. *In vitro* DNA products were synthesized from the respective viral RNA templates by use of the recently discovered RNA-dependent DNA polymerase. By reciprocal RNA-DNA hybridization experiments large differences in the genetic information of the two viruses were demonstrated.

3. *In vitro* hemopoietic culture systems which should prove useful for the study of murine leukemia viruses have been developed.

4. Evidence has been obtained which indicates that cells transformed by murine sarcoma virus but which are free of infectious virus lack detectable transplantation antigens.

5. Genetic factors were studied which affect the inducibility of C-type RNA viruses from embryo cultures of mouse strains with high and low incidence of spontaneous leukemia. Both virus inducibility and the capacity of the virus to persist in cells from which it was activated were found to be inherited as dominant genetic characteristics. The results further indicated that the factors controlling virus activation and persistence in culture also play an important role in spontaneous virus expression in the animal.

Significance to Biomedical Research and the Program of the Institute:

Genetic studies of temperature sensitive mutants of murine leukemia viruses should provide insight into mechanisms of replication and transformation by viruses of the leukemia-sarcoma complex. An understanding of the genetic parameters regulating induction of endogenous virus of murine cells should eventually lead to methods of establishing whether human cells also contain endogenous C-type viruses.

Proposed Course:

To examine, by a genetic approach, mechanisms of C-type oncogenic virus replication and induction.

Honors and Awards:

None

Publications:

Stephenson, J.R., Axelrad, A.A., McLeod, D.L. and Shreeve, M.M.: Induction of colonies of hemoglobin-synthesizing cells by erythropoietin in vitro. Proc. Nat. Acad. Sci. U.S.A. 68: 1542-1546, 1971.

Stephenson, J.R. and Aaronson, S.A.: Murine sarcoma and leukemia virus: genetic differences determined by RNA-DNA hybridization. Virology 46: 480-484, 1971.

Stephenson, J.R. and Aaronson, S.A.: Antigenic properties of murine sarcoma virus transformed Balb/3T3 nonproducer cells. J. Exper. Med. In press.

Stephenson, J.R., Reynolds, R.K. and Aaronson, S.A.: Isolation of murine leukemia virus conditional lethal mutants. Virology. In press.

Stephenson, J.R., Scolnick, E.M. and Aaronson, S.A.: Genetic stability of the sarcoma viruses in murine and avian sarcoma virus transformed nonproducer cells. Int. J. of Cancer. In press.

Serial No. NCI-4857
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Physical and Chemical Properties of the Reverse Transcriptases of RNA Tumor Viruses

Previous Serial Number: None

Principal Investigator: Dr. Steven R. Tronick

Other Investigators: Dr. Stuart A. Aaronson
Dr. Edward M. Scolnick
Dr. George J. Todaro

Cooperating Units: Inside NIH:
Dr. Wade P. Parks, VCB, NCI

Outside NIH:
Meloy Laboratories, Inc., Springfield, Virginia

Man Years:

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description

Objectives:

1. To determine the molecular weights of the polypeptide subunit(s) of murine leukemia virus reverse transcriptase.

2. To determine which enzymatic activities are associated with the subunits.

3. To study the reversible denaturation of the enzyme by guanidine hydrochloride (GuHCl).

4. To determine whether reverse transcriptases from non-mammalian RNA tumor viruses and the DNA polymerases from normal mammalian cells can be reversibly denatured by (GuHCl).

Methods Employed:

Standard biochemical techniques for assaying DNA polymerase, denaturing enzymes, and determining molecular weights of proteins.

Major Findings:

Rauscher-MuLV reverse transcriptase is denatured by, and renatures upon removal of GuHCl. The enzyme is composed of one polypeptide chain of molecular weight approximately 70,000 - 80,000. The renatured enzyme obtained from an agarose column run in 6M GuHCl and reducing agent still possesses RNA and DNA directed DNA polymerase activities associated with the native enzyme. The renatured enzyme reacts with antibodies specific for the native enzyme. The enzyme is optimally renatured in the presence of sulfhydryl compounds, 0.3M salt, and Triton X-100 or bovine serum albumin. The enzyme from feline leukemia virus behaves similarly to RMuLV; however, the enzyme from Rous sarcoma virus does not renature under these same conditions.

Significance to Biomedical Research and the Program of the Institute:

Detailed characterization of tumor virus DNA polymerases is important for understanding the mode of replication of these viruses in mammalian cells and for understanding possibly the mechanism of the malignant transformation of cells by these viruses.

Proposed Course:

The reversible denaturation by GuHCl of viral reverse transcriptases will be studied in more detail. Attempts will be made to irreversibly denature host cell DNA polymerases, but not the viral enzyme. If successful, this will provide a rapid, specific assay for virus in host cells.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4858
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: A. Identification of human RNA tumor virus

B. Mechanism of transformation of RNA tumor viruses

Previous Serial Number: NCI-4837

Principal Investigator: Dr. Edward M. Scolnick

Other Investigators: Dr. David M. Livingston
Dr. Raoul Benveniste
Dr. Jeffrey Ross
Dr. Stuart A. Aaronson
Dr. George J. Todaro

Cooperating Units: Inside NIH
Dr. Wade Parks, VCB, NCI

Outside NIH
Meloy Laboratories, Inc.
Springfield, Virginia

Man Years:

Total:	1.1
Professional:	1.0
Other:	0.1

Project Description

Objectives:

Project A

We have developed three ways in the past year of identifying the expression of C-type viruses in cells. They are:

1. The viral reverse transcriptase provides a sensitive catalytic assay for the presence of virus in cells. Sensitive assays have been developed for its detection. Special use has been made of synthetic polymers since they are excellent templates for all known viral reverse transcriptases. The ratio of activities with different polymers allows one to distinguish

all known viral polymerases from cellular or mycoplasma DNA polymerases. Further identification of a polymerase as viral can be made if an antibody to the viral enzyme is available. Antibodies to mouse and cat viral reverse transcriptases have been prepared. These antibodies do not inhibit cellular DNA polymerases, but do inhibit the viral enzyme from infected cells. The reverse transcriptases of mammalian C-type viruses of the mouse, cat, rat, or hamster are all inhibited by these antibodies. However, the reverse transcriptases of two primate C-type viruses from a Woolly monkey and Gibbon ape are not inhibited. Therefore, further sera are being prepared against these latter polymerases to obtain a reagent which hopefully will cross react with a potential human C-type virus. Using this methodology one C-type virus growing in human cells, ESP-1, has been shown to be a contaminating mouse virus. Another, RD-114, is as yet not classifiable by these methods.

2. The internal 'gs' antigen of C-type viruses also has interspecies determinants with sera which detect these determinants; a search for this protein in human tumors can be made. To this end, with Dr. Wade Parks, a sensitive radioimmunoassay has been set up to search for the interspecies antigen in tumors.

For both proteins, polymerase and internal antigen, in addition, solid phase purification schemes have been devised by coupling their specific antibodies to sepharose. Results of this affinity chromatography show that each of these can be purified from crude cell extracts in highly concentrated form using this approach. When coupled with the assays available for them, if either is present in any human tumor, it should be possible to identify it.

3. In certain classes of RNA tumor virus transformed nonproducer cells, there is no evidence for either internal antigen or viral reverse transcriptase. However, viral-specific RNA can still be detected using RNA-DNA hybridization techniques. A highly labelled DNA copy of viral RNA can be prepared and used as a probe for RNA in cells. Viral RNA has been detected in such nonproducer cells and is being characterized. A rapid sensitive assay for detection of the hybrid has been devised using enzymes which degrade single-stranded DNA but not RNA-DNA hybrids. Using this assay and DNA made from the RNA of primate C-type viruses attempts are in progress to identify viral-specific RNA in human tumors and to correlate the results with those of the other assays.

Project B

To study the mechanism of transformation by RNA tumor viruses, temperature sensitive mutants of RNA sarcoma viruses have been isolated. Three mutants which transform cells at 32° have been isolated. These cells revert to normal at the nonpermissive temperature 39.5°C. Studies are in progress to identify the number of genes involved and what their products are.

Methods Employed:

Project A

The methods involved in studies on the reverse transcriptase and gs antigens are column chromatography, ampholine isoelectric focusing, polyacrylamide gel electrophoresis, radioimmunoassays, complement fixation and immunodiffusion. To study hybridization a new enzymatic method is being worked out.

Project B

To study sarcoma mutants, standard tissue culture techniques are used. In addition, a method for replica plating cells is also used.

Major Findings:

Project A

By studying the immunology of the reverse transcriptase we have identified different classes of C-type viruses. This has been confirmed with radioimmunoassay of the gs antigens. Lower mammalian C-type viruses can be distinguished from primate C-type viruses. Thus, possible human isolates can be identified as primate in origin.

Project B

Three mutants of mammalian sarcoma virus have been isolated. They are being studied to try to determine the mechanism of transformation.

Significance to Biomedical Research and the Program of the Institute:

Project A

This project is directly related to attempts to identify an RNA tumor virus as a causative agent in human cancer.

Project B

This project is to determine how such a virus causes cancer and, hopefully, thus to cure it.

Proposed Course:

Project A

This project will continue as detailed above.

Project B

This project will continue as detailed above.

Honors and Awards:

None

Publications:

Aaronson, S.A., Parks, W.P., Scolnick, E.M., and Todaro, G.J.: Antibody to the RNA-dependent DNA polymerase of mammalian C-type RNA tumor viruses. Proc. Nat. Acad. Sci. 68: 920-924, 1971.

Ross, J., Scolnick, E.M., Todaro, G.J., and Aaronson, S.A.: Separation of murine cellular and murine leukemia virus DNA polymerases. Nature 321: 163-167, 1971.

Caskey, C.T., Scolnick, E.M., Topkins, R., Milman, G., and Goldstein, J.: Release factors: In vitro assay and purification. Methods in Enzymology 20: 367-375, 1971.

Scolnick, E.M.: Protein biosynthesis in bacterial systems. Laskin, A.I. and Last, J.A. (Eds.): Methods in Molecular Biology. New York, Marcel Dekker, Inc., 1971, pp. 201-212.

Aaronson, S.A., Todaro, G.J., and Scolnick, E.M.: Induction of murine C-type viruses from clonal lines of virus-free Balb/3T3 cells. Science 174: 157-159, 1971.

Scolnick, E.M.: RNA-dependent DNA polymerases in mammalian cells. Develop. Biol. 76: 175-176, 1971.

Scolnick, E.M., Parks, W.P., Todaro, G.J., and Aaronson, S.A.: Primate C-type viral reverse transcriptases: Immunological properties. Nature New Biol. 235: 35-40, 1972.

Ross, J., Haviv, C., Scolnick, E.M., and Leder, P.: In vitro transcription of globin M-RNA by avian myeloblastosis reverse transcriptase. Proc. Nat. Acad. Sci. 69: 264-268, 1972.

Livingston, D.M., Scolnick, E.M., Parks, W.P., and Todaro, G.J.: Affinity chromatography of murine viral reverse transcriptase. Proc. Nat. Acad. Sci. 69: 393-397, 1972.

Parks, W.P., Scolnick, E.M., Ross, J., Todaro, G.J., and Aaronson, S.A.: Immunological relationships between viral reverse transcriptase. J. Virol. 9: 110-115, 1972.

Scolnick, E.M.: RNA-dependent DNA polymerases of RNA-containing viruses. Current Topics in Biochemistry, Academic Press. In press.

SUMMARY

Annual Report of the Viral Biology Branch, Viral Oncology
Etiology, National Cancer Institute
July 1, 1971 through June 30, 1972

The Viral Biology Branch conducts research on viral and host factors related to the carcinogenic process and on the development and evaluation of measures for control in experimental systems. Specifically, investigations are conducted to identify virological activity in oncogenesis. In defined systems, the biological interaction between viruses co-infecting the host is studied to determine the effect on tumor virus expression and on the development and course of disease in the host. Ultrastructural studies permit detection and morphological characterization of viruses associated with disease processes and the effect of viral activity on internal organization of the cell. Biochemical events related to viral infection are followed. Investigations continue on alterations in the surface membrane of cells that undergo neoplastic transformation and the immune responses which may be induced in the host against new membrane antigens of neoplastic cells. Combined chemotherapeutic and immunological measures are evaluated as approaches to the control of viral expression and the growth of tumor cells.

The Office of the Chief coordinates the research efforts conducted in the Sections within the Branch and collaborative studies with investigators in other laboratories. The Chief serves as Chairman of the Developmental Research Segment of the Special Virus Cancer Program. In this capacity he has been responsible for the administration and management of 29 extramural research contracts within the Segment program concerned with: the detection, isolation, and characterization of viruses associated with tumors in man and animals; molecular biological studies on virus-host cell relationships; the activity of enzymes within virions as these may be related to the processes of virus infection, integration, replication and cell transformation; virus nucleic acid base sequences; immunological factors activating virus expression in covert infections; evaluation of immunological approaches to the control of tumor virus infection or expression; maintenance and use of non-human primates for the study of viruses of possible etiological importance in human neoplasia; and the production of viruses and cells in quantity in support of the overall Special Virus Cancer Program. He is supported by the Vice Chairman of the Segment, secretarial assistance, and investigators within and outside the Branch who serve as Project Officers and/or members of the working contract review group. He has been apprised of developments in molecular virology by an advisor for molecular studies functioning within his office.

The Cell Biology Section specializes in investigations on the role of the tumor cell surface membrane in carcinogenesis and in the induction of cell-

mediated host immune responses to tumor cell antigens. The Virus and Disease Modification Section conducts a multi-disciplinary approach to the detection, prevention, treatment and/or control of neoplastic disease in experimental animal systems, and studies in vitro and in vivo on the interactions between oncogenic and non-oncogenic viruses which may affect the disease process. The Human Tumor Studies Section is concerned with the study of viruses associated with, or contributing to further knowledge of possible etiological relationships in, human cancer. The Microbiology Section seeks to obtain information on biological interactions in selected tumor virus-cell systems. The Experimental Pathology Section follows the progress of viral infection, immune responses, and the influence of the immune state on the development of neoplastic lesions in selected animal systems. The Electron Microscopy Section devotes its efforts to the detection of particulate viruses in tissues or cell cultures, the definition of ultrastructural characteristics of viruses and of intracellular changes associated with virus infection, the localization of antigens within cells, and support to other investigators requiring such services.

Research Developments within the Branch:

An increase in the immunogenicity of weak tumor cell surface antigens was obtained by incorporating influenza virus antigens into the cell surface membranes. Following formalin treatment to inactivate the influenza virus, vaccination of animals with homogenates of these cells gave significant protection to animals against challenge with viable cells of that tumor. These original observations were confirmed and extended to demonstrate induced immunity to SV40 transformed tumor cells and methylcholanthrene-induced sarcomas.

Dual infection of cells by an oncogenic RNA virus and a non-oncogenic virus may enhance the oncogenic response. Investigations conducted in vivo and in vitro have shown that infection by an arbovirus stimulated the production of increased amounts of mouse sarcoma virus. Somewhat similar results were obtained when a non-virus-producing line of SV40 transformed cells was infected with mouse sarcoma virus. Evidence showed activation of SV40 production, while the MSV(M) released in this line induced an altered pathology in mice. Arbovirus infection also appears to affect neoplastic cells. A lymphoblastic cell line established from a spontaneous lymphoma of the AKR strain mouse lost Gross virus cell membrane antigen and its tumorigenicity after infection with Germiston virus.

Previous exposure of mice to BCG followed by infection with MSV(M) in admixture with BCG completely inhibits tumor development. Lymphocytes from these animals, while not as effective as lymphocytes from tumorous animals, were capable of killing labeled MSV(M)-induced tumor cells as demonstrated by release of Cr 51. Studies on BCG and another non-specific immune stimulator, Corynebacterium granulosum, were extended

to animals which had been inoculated with the transplantable murine lymphoid leukemia, LSTRA, and subsequently placed in remission by treatment with BCNU. In groups of animals treated with the immune stimulators prior to relapse, 70 to 80 percent remained disease-free as compared to the 80 to 90 percent mortality observed in those groups that received only drug treatment. Similar groups of mice in remission following treatment with 25 ml/Kg of BCNU received single or multiple injections of spleen cells from normal and immune mice. All groups contained a high number of survivors. Why normal spleen cells were effective is not known. Prolonged host survival was not obtained when immune and normal spleen cells were inoculated after treatment with 20 ml/Kg of body weight of BCNU, suggesting a reduced tumor burden is essential.

An antibiotic, Streptonigrin, is highly effective in nonogram amounts in inhibiting murine leukemia and sarcoma viruses in vitro. A marked decrease in titer of Rauscher MLV recoverable from infected mice as well as an increase of survival time of these mice has been observed in groups treated with Streptonigrin. This antibiotic is interesting in that it inhibits the avian myeloblastosis virus reverse transcriptase activity.

In experiments exploring humoral factors affecting tumor growth in animals the effectiveness of immune sera in inhibiting the growth of virally-induced mouse sarcoma cells in animals was shown to correlate with the anti-cell membrane and virus neutralizing titer of a serum. Pretreatment of mice with serum from animals with progressively-growing sarcomas enhanced the growth of a challenge inoculum of tumor cells. This serum possessed no anti-cell membrane, virus neutralizing, or cytotoxic activity. Tumor cell growth was inhibited by sera from animals in which tumor had regressed and by sera from tumor-immune animals of another strain. These sera were not cytotoxic for tumor cells, but had anti-membrane and neutralizing activity. Fifty percent of the serum-treated mice that initially rejected the challenge dose of MSV-induced sarcoma cells rejected a second larger challenge inoculum given 45 days later.

A rat virus of C-type morphology, continuously released by the R-35 cell line established from a spontaneous mammary carcinoma of the Sprague-Dawley rat has been reported to have specific infectivity for cultured rat mammary gland tissue. Infected cells underwent transformation and grew to produce tumor when implanted into rats. A number of rats infected shortly after birth eventually developed tumors of possible mammary gland origin.

Experiments were conducted to define the relationship between this virus and other rat C-type viruses. Infectivity studies showed that animal species other than the rat were refractory to the R-35 virus. Immunofluorescence studies were conducted to determine cross reactions between cell membrane antigens of R-35 cells, cells of the WF-1 line originated from a W/Fu rat, and cells of the RMTL-8 line from a chemically-induced mammary tumor in a Sprague-Dawley rat. All these lines shed C-type virus.

Sera from W/Fu rats bearing WF-1 tumor reacted with WF-1 cell membranes in immunofluorescence tests. Some of these sera reacted with R-35 and RMTL-8 cells. Absorption experiments verified the presence of cross-reacting cell membrane antigens, and preliminary results of neutralization tests provided further evidence that these rat-C-type viruses are antigenically closely related and perhaps identical.

The availability of an animal host susceptible to infection by Epstein-Barr virus (EBV) would substantially aid in the study of this virus. Old World monkeys, such as the Rhesus, African green, Bonnet, and other species were found to possess a high incidence of antibody reactive with EBV-associated antigen in immunodiffusion tests. Apparently, these monkeys are infected by a virus related to EBV. However, no such virus has been isolated from these animals, nor do their lymphoid cells respond to infection by EBV as do human lymphocytes. Antibodies reactive with EBV have not been detected in New World monkeys. Bone marrow cells from the marmoset and the Owl monkey were exposed to infective EBV and monitored for transformation and production of EBV antigens. There was no evidence of infection. Whereas long term propagation of human lymphoid cells in vitro appears to be directly related to preexisting infection by EBV, a cell line of lymphoblasts, established from a carcinogen-induced leukemia of a rhesus monkey free of antibody to EBV, showed no evidence of the presence of antigens reactive with antisera to EBV. Thus, the problem of a suitable experimental animal for EBV studies and the question of the cell site and significance of infection by an EBV-related virus in the species of lower primates studied remains unresolved.

A search was undertaken for the presence of virus-like particulates in purified, concentrated fractions of milk from women from families with a history of cancer. No particulates which could be definitely described as B- or C-type were detected. Particles resembling the M-PMV recovered from a monkey mammary tumor were present in 23 of 308 specimens examined by electron microscopy. The presence or absence of such particulates did not correlate with reverse transcriptase activity in the milk samples.

Attempts have been made to activate virus release from cultured mammary tumor cells from humans and dogs by treatment with reagents known to induce virus production in mouse cells. These methods have been unsuccessful in a limited number of trials.

The Electron Microscopy Section has provided support to different investigators within Viral Oncology. Approximately 1500 specimens have been studied. Collaborative studies with Dr. John Hooks and Dr. D. C. Gajdusek, NINDS, on a new virus isolated from brain cell cultures originated from tissues obtained from a patient with Creutzfeldt-Jakob disease showed the agent to be morphologically similar to M-PMV.

Feline sarcoma virus and an associated helper virus infects cat, bovine, and human cells. A non-virus-producing infected cell system would be useful for detecting viruses in human leukemic tissues, providing helper activity to the defective sarcoma virus genome in cells susceptible to

infection by human virus. Serial passage of the sarcoma virus complex in bovine cells ultimately selected a virus population with an enhanced efficiency of infection for bovine cells as compared to feline cells. Focal areas of transformed cells have been selected and cultured. These lines shed substantial, moderate, or essentially no progeny virus. The value of these poor shedder cells as a detector system for other leukemia viruses is being explored.

During the reporting period, 26 papers, published or in press, have originated in the Branch and members of the staff have been co-authors on 10 papers resulting from collaboration with others. Members of the staff have presented by invitation 8 lectures in this country and abroad.

Other Activities within the Branch:

In addition to their intramural research activities, a number of investigators within the Branch have devoted substantial amounts of their time as Project Officers, as site visitors to laboratories conducting research under contract, and as members of Groups reviewing research contract proposals within the Special Virus Cancer Program. Dr. Michael Chirigos, Associate Chief of the Viral Biology Branch, has replaced Dr. Jack Gruber as Vice Chairman of the Developmental Research Segment. Dr. Gruber has been reassigned to a position of greater responsibility. Dr. Timothy O'Connor has acted as advisor in Molecular Virology to the Associate Scientific Director for Viral Oncology. He has coordinated studies on the inhibition of viral polymerase by rifamycin derivatives in a collaboration between NCI and the Dow Chemical Company, and has assisted in contract reviews as a member of the Developmental Research Segment Working Group. Dr. Charles Boone has served on the Immunology and Epidemiology Segment Working Group. Dr. Gary Pearson has contributed his services as Executive Secretary to the Immunology and Epidemiology Segment. In addition, other members of the Staff serve as Project Officers and participate in monitoring research contract activities.

Serial No. NCI-4874
1. Viral Biology Branch
OASDVO, Etiology
2. Electron Microscopy Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971, through June 30, 1972

Project Title: Ultrastructural Studies on Tissue and Virus in Relation to Neoplastic Diseases

Previous Serial Number: Same.

Principal Investigator: Dr. Harish C. Chopra

Other Investigators: Dr. Herbert K. Oie (NCI-4891)

Cooperating Units: Inside NIH
Dr. Gary Pearson, VBB, NCI (NCI-4880)
Dr. Paul Ebert, VBB, NCI (NCI-4876)
Dr. D. Ablashi, VLLB, NCI (NCI-4834)
Dr. E. Plata, VLLB, NCI (NCI-4828)
Dr. B. Gerwin, VLLB, NCI (NCI-4846)
Dr. Y. Ikawa, VLLB, NCI (NCI-4847)
Dr. J. Hooks, C&FR, NINDS

Outside NIH
Dr. M. Brennen, Michigan Cancer Foundation, Detroit, Michigan
Dr. D. Fine, Bionetics Research Laboratories, Bethesda, Maryland
Dr. M. Mason, Mason Research Institute, Worcester, Massachusetts
Dr. V. Bergs, University of Miami School of Medicine, Miami, Florida
Dr. J. Warren, Germfree Life Research Center, Miami, Florida

Man Years:

Total:	5
Professional:	1
Other:	4

Serial No. NCI-4874

Project Description

Objectives:

1. To acquire information on the association of virus particles detected in the milk of women by electron microscopy with family histories showing a high incidence of cancer.

2. To attempt to isolate viruses from mammary carcinomas and cell cultures.

3. To further investigate the Mason-Pfizer monkey virus (M-PMV), isolated from a monkey breast tumor.

4. To provide electron microscope support to different NCI projects.

Methods Employed:

Biopsy materials, cultured cells and virus concentrates were prepared by thin sectioning and negative staining techniques of electron microscopy. Tumor tissues were cultured and examined by electron microscopy for virus detection. The cultured cells were co-cultivated or subjected to various carcinogens for virus activation and EM detection. Electron microscopic observations were interpreted, evaluated, and suitably recorded.

Major Findings:

1. Electron microscopic search for virus or virus-like particles in the purified and concentrated fractions of milk from women from families with a history showing a high incidence of cancer was undertaken. The milk samples from women both with and without family history of cancer were processed according to Dr. Dan Moore's procedure for concentration of the particulate material and examination by the negative staining technique of electron microscopy. An attempt was made to correlate the occurrence of virus-like particles with the presence of RNA dependent DNA polymerase in these milk samples. Electron microscopic investigations have revealed only suspicious virus-like particles and no definite virus of either type B or type C. However, 23 milk samples of 308 examined in our blind study have shown the presence of particles morphologically resembling the negatively stained virus (M-PMV) isolated from the monkey breast tumor. Eighteen of these milk samples demonstrating virus-like particles were from patients that had family history of cancer, i.e., five with a breast cancer family history, and thirteen with other types of cancer. No significant results were obtained correlating the presence of particles with the presence of RNA dependent DNA polymerase activity. Further study is in progress to determine the viral nature of the particles detected in the milk samples.

2. Extensive investigations to detect and isolate a viral agent from the human breast biopsies and from dog mammary tumors have been in progress. Tissue samples and cultured human breast tumors (in collaboration with Dr. Plata) were examined in thin section. No virus particles were detected in human breast material. Primary cultures are being subjected to various chemical carcinogens such as IUdR and BUdR with or without DMSO to activate virus production. No virus was detected in the dog mammary tumors examined. Three dog mammary cell cultures are being maintained in this laboratory, and various experimental approaches are being undertaken to activate or rescue viral particles.

Isolation and morphological characterization of a new virus from a human brain cell culture grown in vitro and spontaneously transformed was undertaken in collaboration with Dr. John Hooks and Dr. D. C. Gaidusek of the Institute of Neurological Diseases and Stroke, NIH. Virus particles, morphologically resembling the known oncogenic RNA viruses and monkey tumor derived virus were observed in the transformed cells in this culture. Intracellular particles measuring about 60-80 nm in diameter were observed within the cytoplasm which appeared to be incorporated into the budding forms of the virus particles. The extracellular virus, measuring 100-120 mu, contained an outer unit membrane and an inner diffused nucleoid. Although the transformed cells, when inoculated subcutaneously into newborn hamsters, have induced tumors, the oncogenic potential of the cell-free virus still remains to be demonstrated.

3. Further studies on morphological characterization of the virus (M-PMV) isolated from monkey breast tumor were conducted. Longterm propagation of this virus in human leukocytes (NC-37) has demonstrated, in general, a morphology resembling that of type B virus particles. As observed in the original tumor, the virus particles replicated in the cytoplasm as intracytoplasmic type A particles which were released extracellularly from the cell surface by the budding process. However, the extracellular virus particles in NC-37 cell cultures have demonstrated eccentric nucleoids, a characteristic feature of type-B particles of mouse mammary tumor virus (MTV).

Investigations on the transforming ability of (M-PMV) virus, and its in vivo oncogenic potentials were carried out in collaboration with investigators at the Bionetics Research Laboratories. This agent has been found to produce a characteristic syncytial type cytopathic effect on the monkey and human foreskin cell cultures (Fine et al, 1971). The multinucleated cloned cells from these cultures have fulfilled several criteria of in vitro transformation, and are continuously producing infectious virus. The transformed cells were inoculated by various routes into newborn male and female rhesus monkeys. Electron microscopy of the tissue biopsied from the cellular mass that developed at the site of inoculation and from adjacent lymph nodes has revealed the presence of (M-PMV) virus particles. Further in vitro and in vivo studies of (M-PMV) agent are in progress.

4. The Electron Microscopy Section has provided support to various investigators within the Viral Oncology area. Several collaborative research projects, reported previously, were completed this year and the reports have either been published or submitted for publication.

Significance to Biomedical Research and the Program of the Institute: Ultrastructural studies on tissues and viruses in relation to neoplastic diseases have contributed significantly to the virus-cancer program, especially for virus detection in human neoplasia. Further collaborative investigations with other NCI investigators have provided necessary support on the ultrastructural aspects of various research projects.

Proposed Course:

This activity will be maintained.

Honors and Awards:

None.

Publications:

Chopra, H.C.: Oncorna type virus particles in a tumor of a rhesus monkey. Bibliotheca Haematologica, S. Karger (In press).

Chopra, H.C. and Oie, H.K.: Possible etiological role of virus particles detected in rat and monkey mammary carcinomas. J. Nat. Cancer Inst. (In press).

Chopra, H.C., Hooks, J.J., Walling, M.J., and Gibbs, C.J., Jr.: Morphology of simian foamy viruses with particular reference to virus isolated from spontaneous tumor of a rhesus monkey. J. Nat. Cancer Inst. 48: 451-463, 1972.

Chopra, H.C., Lloyd, B.J., Jr., Ablashi, D.V., and Armstrong, G.R.: Morphologic studies of a cytomegalovirus isolated from an owl monkey. J. Nat. Cancer Inst. (In press).

Chopra, H.C., Zelljadt, I., Woodside, N., and Walling, M.J.: Studies on virus particles resembling oncogenic RNA viruses in a monkey breast carcinoma. Cancer 28: 1406-1415, 1971.

Mason, M.M., Bogden, A.S., Ilievski, V., Esber, H.J., Baker, J.R., and Chopra, H.C.: Clinical history of an adenocarcinoma bearing rhesus monkey with a virus resembling oncogenic RNA viruses. J. Nat. Cancer Inst. (In press).

Sarkar, N., Moore, D., Kramarsky, B., and Chopra, H.C.: The mammary tumor virus in An Atlas on Ultrastructure of Viruses. ed. Dalton, A.J. and Haguenau, F. Academic Press (In press).

Presentations:

Dr. Chopra presented a paper entitled: Further studies on virus particles resembling oncogenic RNA viruses in monkey mammary adenocarcinoma at the Second National Conference on Breast Cancer, Los Angeles, California, 1971.

Dr. Chopra presented a paper entitled: Possible etiological role of virus particles detected in rat and monkey mammary carcinomas at the Symposium on Mammary Neoplasia, Cherry Hill, New Jersey, 1971.

Dr. Chopra presented a paper entitled: Oncorna type virus particles in a tumor of a rhesus monkey at the V International Symposium on Leukemia Research, Padova, Italy, 1971.

Dr. Chopra presented a paper entitled: Comparative study of simian foamy viruses and monkey tumor derived virus at Cold Spring Harbor Symposium, Long Island, New York, 1971.

Serial No. NCI-4875
1. Viral Biology Branch
OASDO, Etiology
2. Virus & Disease Modification Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Control of Oncogenic Viruses and Induced Diseases Mediated by External Factors

Previous Serial Number: Same

Principal Investigator: Dr. Michael A. Chirigos

Other Investigators: Dr. Wilna Woods (NCI-4877)
Dr. Donald Schwartz (NCI-4879)
Dr. John Pearson (NCI-4878)
Dr. Gary Pearson (NCI-4880)

Cooperating Units:

Inside NIH:
Dr. S. Chaparas, LBP, DBS
Dr. Jerry Rice, BB, NCI
Dr. Tom Cameron, CCNSC, NCI

Outside NIH:
Dr. G. Spahn, Microbiological Associates, Inc., Bethesda, Maryland
Dr. L. S. Rabstein, Microbiological Associates, Inc. Bethesda, Maryland
Dr. Robert Rutman, University of Pennsylvania, Department of Biochemistry, Philadelphia, Pennsylvania
Dr. E. Jensen, Hazleton Laboratories, Falls Church, Virginia
Dr. Windsor Cutting, University of Hawaii Medical School, Honolulu, Hawaii

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description

Objectives:

The overall aim of this project is to determine the most effective means of preventing and/or controlling leukemias and sarcomas. Several therapeutic

approaches are examined in animal model systems to assess the most feasible approach that can be employed which will exert maximum antitumor activity, with the least deleterious effect on normal host factors. Host factors which may be affected by therapy are immuno-competence, organ and/or cell toxicity, kidney or liver damage.

Methods Employed:

A. Virus and Tumor Animal Systems.
The following are employed for in vitro and in vivo studies.
1. The Rauscher, Friend, Moloney, Graffi and Gross leukemogenic viruses, and the Moloney, Harvey and PV sarcomas.
2. AKR mice with spontaneous lymphoma.
3. Rat Gross, Nova and Dunning Leukemias.
B. Humoral and cellular antibodies as measured by standard procedures or new methods developed by Section staff.
C. In vitro and in vivo assays for measuring viral replication.

Major Findings:

1. Inhibitors: Several antibiotics, drugs and plant extracts were tested for antiviral and/or antitumor activity. Alkaloid extracts of (crystalline and residual alkaloid fractions) Narcissus bulbs were found to be highly inhibitory in vitro against RLV, MLV and AKR with relatively little to no cellular toxicity. The antibiotic, Streptovaricin A & C fractions were also inhibitory to MLV and RLV in vitro. This antibiotic was reported to have inhibitory activity on the MLV reverse transcriptase. Neither of these compounds were found to have marked in vivo antiviral activity. Streptonigrin (SN) another antibiotic, was found to possess a highly inhibitory activity in vitro against MLV, RLV, AKR leukemia viruses as well as the MSV(M) virus. Of particular significance is that it was highly effective in nanogram amounts. At 10 nanogram per ml a greater than 95% inhibition of viral replication was attained with little to no inhibition of normal cellular RNA or DNA synthesis nor evidence of cellular toxicity.

Streptonigrin was also found to exert excellent antiviral and antitumor activity in vivo. Treatment of viremic mice, infected with RLV, resulted in marked decrease in virus titer (>3 logs) and a substantial increase in survival time (>100%). Streptonigrin used prophylactically and therapeutically effectively retarded MSV(M) induced tumors. Of particular significance was its excellent inhibitory activity on AMV reverse transcriptase. A 100% inhibition of enzyme activity was attained with 175 µg of SN. Relatively few agents possess all three characteristics of in vitro, in vivo, and enzyme inhibitory activity demonstrated by SN.

2. Immuno-stimulators: Three non-specific RES stimulators were tested for their capacity to retard or depress spontaneous AKR lymphoma (BCG, Poly I:C and pyran copolymer). Preliminary results indicate BCG and pyran copolymer when administered during the period of active disease results in a retardation of lymphoma symptoms. This was reflected by a significant extension in

survival time over untreated controls.

Qualitative and quantitative studies with BCG (different preparations) indicate that specific factors are required to effectively stimulate the RES. The most important to date are: viable organisms vs. dead; requirement for greater than 10^5 organisms, and i.d. route. Micro-methods are being developed to measure BCG lymphocyte stimulation.

3. Virus potentiation: In previous reports innocuous viruses (LDV and Guaroa Arbovirus) were shown to potentiate murine leukemia and sarcoma viruses. Of particular significance was a 90% increase in tumor incidence, infection and death which was attained in mice co-infected with LDV and MSV(H). Preliminary histological indicate that this response may be due to a transitory depletion of thymic dependent cells caused by LDV infection.

4. Lewis lung tumor: A transplantable lung tumor mouse model system has been established. This solid tumor will serve as a model for studies on delayed hypersensitivity for future immuno-therapy trials.

Significance to Biomedical Research and the Program of the Institute:

The multi-disciplinary approach represented in this project is almost entirely directed towards the prevention, treatment and control of cancer. It is necessary to work with animal systems to gain basic information which is ultimately applied to the control of human neoplasia. The greatest strides made in controlling cancer are with drug therapy. However, chemotherapy has several serious limitations. Studies concerned with immunotherapy and immuno-stimulants, described herein, indicate them to be effective control measures particularly in combination with drug therapy. Non-drug therapeutic measures are presently at hand, and if better understood and judicially applied, can serve to better control human cancer.

Proposed Course:

Each of the areas described under objectives will be pursued. More emphasis will be placed on quantitative and qualitative studies on three specific areas, i.e., Inhibitors, Immune-Stimulators and Virus Potentiation. Biochemical parameters will be investigated with the active inhibitors to assess mechanism(s) of action. In vitro assays will be employed to assess whether the immuno-stimulatory activity demonstrated by BCG, Pyran and Poly I:C is of humoral or cellular type.

Further studies will be pursued in virus potentiation. It will be important to determine whether infection by an innocuous virus causes depletion of specific cells (thymus dependent cells) thus making host tissue and/or organs more prone to infection, replication and transformation by low oncogenic virus input.

Other Activities:

Served as Chairman of the Special Animal Leukemia Ecology Studies Segment up to November 1971, at which time the Segment activities were reorientated. Serves as Vice-Chairman of the Developmental Research Segment of the SVCP and Project Officer or Assistant Project Officer on 15 contracts in the SVCP.

Honors and Awards:

Dr. Chirigos participated in a round table discussion on "Unifying concepts of leukemia and related neoplasms in man and animals leading to eventual control and prevention" and also participated in the final round table summation of the International Symposium on Leukemia Research held under the auspices of the World Committee for Comparative Leukemia Research and the World Health Organization.

Dr. Chirigos, by invitation, presented lectures regarding the most recent developments on cancer research, at the following institutes in Greece: Saint Savas Hospital, Peraus Hospital, and the University of Athens.

He presented, by invitation, a lecture on "Virus-Tumor Complex" at the Tri Beta Biological Society at Western Maryland College.

Publications:

Chirigos, M. A.: Comments on treatment of feline leukemia. The Jour. of the Amer. Vet. Med. Assoc. 158: 1137, 1971.

Turner, W., Ebert, P. S., Bassin, R., Spahn, G., and Chirigos, M. A.: Potentiation of murine sarcoma virus (Harvey)(Moloney) oncogenicity in lactic dehydrogenase-elevating virus-infected mice (35482). Proc. Soc. Exp. Biol. and Med. 136: 1314-1318, 1971.

Rice, J. M., Turner, W., Chirigos, M. A., and Spahn, G.: Dose responsiveness and variation among inbred strains of mice in production of interferon after treatment with Poly(I)·Poly(C)[Poly-D-Lysine] complexes. Appl. Micro. 22: 380-386, 1971.

Schwartz, D. B., Zbar, B., Gibson, W. T., and Chirigos, M. A.: Inhibition of murine sarcoma virus oncogenesis with living BCG. Int. J. Cancer 8: 320-325, 1971.

Massicot, J. G., Woods, W. A., and Chirigos, M. A.: Cell line derived from a murine sarcoma virus (Moloney Pseudotype)-Induced tumor: Cultural, Antigenic, and Virological Properties. Appl. Micro. 22: No. 6, p. 1119-1122, 1971.

Turner, W., Ebert, P., Riechers, L., Pearson, J. W., and Chirigos, M. A.: Elucidation of the nature of the murine oncogenic virus inhibitor isolated from JLS-V5 cell line (36044). Proc. Soc. Exp. Biol. and Med. 138: 1030-1034, 1971.

Chirigos, M. A., Pearson, J., Spahn, G., and Rutman, R.: Current studies on oncorna virus therapy. Vth International Symposium on Comparative Leukemia Research. Padova-Venice (In Press).

Pearson, J. W., Pearson, G. R., Gibson, W. T., Chermann, J. C., and Chirigos, M. A.: Combined chemo-immunostimulation therapy against murine leukemia. Cancer Research. (In Press).

Woods, W. A., Turner, W., and Chirigos, M. A.: Co-infection of mouse spleen cells with murine sarcoma virus and guaroa virus. Appl. Micro. (In Press).

Serial No. NCI-4876
1. Viral Biology Branch
OASDVO, Etiology
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Biochemical Alterations Occurring in Host Tissue as a Result of Oncogenic Virus Infection and Tumor Induction.

Previous Serial Number: Same

Principal Investigator: Dr. Paul S. Ebert

Other Investigators: None

Cooperating Units: Inside NIH

Dr. M. A. Chirigos, VBB, NCI (NCI-4875)
Dr. Willie Turner, VBB, NCI (NCI-4889)
Dr. John Pearson, VBB, NCI (NCI-4878)
Dr. Gary Pearson, VBB, NCI (NCI-4880)
Dr. H. Chopra, VBB, NCI (NCI-4874)
Dr. Brenda Gerwin, VLLB, NCI (NCI-4846)
Dr. V. Dunkel, VBB, NCI (NCI-4881)
Dr. S. Margolis, VBB, NCI (NCI-4882)
Dr. E. Chan, VBB, NCI, Visiting Scientist (NCI-4894)
Dr. D. P. Tschudy, Metabolism Branch, NCI
Dr. E. Weinbach, LPD, NIAID
Dr. D. N. Buell, GL&C, NCI
Dr. Y. Ikawa, VLLB, NCI, Visiting Scientist (NCI-4847)

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

1. To investigate the possible viral etiology of breast cancer by testing human milk for the presence of RNA-directed DNA polymerase (reverse transcriptase).

2. To investigate the possible relationship between virus titer and δ-aminolevulinic acid (ALA) synthetase activity and extend this finding to other oncogenic viruses and cell lines.

3. To determine the significance of the heme biosynthetic pathway in non-erythroid cell lines.

4. To investigate the process of differentiation by following enzymatic changes in cells which differentiate along the erythoid pathway when exposed to dimethylsulfoxide (DMSO).

5. To delineate the biochemical defect(s) in the genetic disease acute intermittent porphyria (AIP).

Methods Employed:

Conventional biochemical procedures were utilized to observe the alterations occurring in normal and virus-infected cells in culture. ^{14}C-ALA production in tissue culture cells was quantitatively measured by passage of incubation mixtures through Dowex 50 column to selectively remove radioactive substrate and metabolites in one step. Virus particles from human milk samples were concentrated and purified by centrifugation and standard density gradient techniques. Reverse transcriptase was measured by techniques described by Spiegelman et al. Briefly, virus particles were disrupted by detergent treatment, incubated with three non-radioactive deoxyribonucleotides and one radioactive deoxyribonucleotide, and the acid-precipitable product of the reaction was collected on Millipore filters and counted in a liquid scintillation counter.

Major Findings:

1. Reverse transcriptase activity in human milk: As part of a pilot project to determine if human breast cancer has a viral etiology, normal milk and milk samples from patients with breast cancer were assayed for reverse transcriptase activity. Using Schlom and Spiegelman's assay for reverse transcriptase in milk (Nature 231: 97, 1971), we could not duplicate the excellent correlation between the presence of viral particles and transcriptase activity as observed by Moore and Spiegelman. Although some correlation was observed, transcriptase activity could be demonstrated in milk samples in which no virus particles were observed by electron microscopy (EM) and vice versa. By further purification of the solublized transcriptase by passage through phosphocellulose columns, some of the milks which were positive by the Spiegelman procedure showed positive DNA polymerase activity either with and without RNA-directed DNA polymerase activity. Attempts to duplicate Schlom and Spiegelman's procedure to isolate high molecular weight RNA:DNA hybrid (Sci. 175: 542, 1972) have been successful using Rauscher leukemia virus, but the method has not yet been applied to the analysis of human milk.

2. Heme biosynthetic activity in virus-containing cell lines: Four fibroblastic cell lines derived from rats were graded according to content of C-type virus particles by EM and immunofluorescence. ALA synthetase activity was found to be proportional to the number of virus particles. Fibroblasts originating from normal W/Fu rat embryos showed very low but detectable levels of the enzyme. The ESP-1 line of cells purportedly containing a human virus showed elevated levels of ALA synthetase activity. A cell line containing Friend virus which can be induced to differentiate along the erythroid pathway showed a high level of ALA synthetase activity. Twenty hours following the addition of DMSO to the media to stimulate erythroid differentiation, the level of ALA synthetase was elevated above the resting level.

3. Biochemical defect(s) in acute intermittent porphyria (AIP): Skin biopsies from seven patients with AIP and six normal persons were grown in tissue culture for 5-10 months. The activity of ALA synthetase porphobilinogen (PBG) deaminase, and enzymes in the electron transport chain (ETC) were compared in the two groups of cultured fibroblasts. Possibly because only low levels of ALA synthetase could be detected in human fibroblasts, no differences in activity could be detected between the two groups, although a distinguishing feature of AIP is elevated levels of ALA in the blood. PBG deaminase activity was decreased in fibroblasts from prophyric patients compared to normal fibroblasts. Enzymatic activities in the ETC of the two groups were examined using the oxygen electrode to determine if any change existed in the sensitivity to inhibitors. Most of the samples of fibroblasts derived from patients afflicted with AIP did not exhibit the typical rotenone inhibition of DPNH dehydrogenase, suggesting the possible presence of a genetic abnormality in the ETC.

Significance to Biomedical Research and the Program of the Institute:

The screen for RNA-directed DNA polymerase in human milk in conjunction with EM studies provides support for broader studies on the relationship of viruses to the etiology of human breast cancer.

Studies on ALA synthetase and heme biosynthetic activity in virus-infected tissue culture cells could reveal an altered requirement of certain viruses or transformed cells for heme-containing compounds such as cytochromes and peroxidases. Investigations of early enzymatic changes during differentiation of primitive cells along the erythroid pathway should lead to a better understanding of the process of differentiation.

Studies of cultured fibroblasts from patients with AIP could delineate the biochemical defects in this genetic disease and could suggest ways to correct this deficiency.

Proposed Course:

Erythroid cell lines will be further investigated to determine what factors are responsible for differentiation along the erythroid pathway. Different inducers of ALA synthetase will be compared with regard to their ability to induce erythroid differentiation.

The observation of the relationship between ALA synthetase activity and virus titer in the rat cell lines will be extended to other cell lines containing different oncogenic viruses.

The pilot project to study reverse transcriptase in human milk will be continued until it can be established whether or not an association exists between the presence of virus particles in milk and breast cancer history.

Honors and Awards: None

Publications:

Ebert, P. S., Maestri, N. E., and Chirigos, M. A.: Erythropoietic responses of mice to infection with Rauscher leukemia virus. Cancer Res. 32: 41-47, 1972.

Serial No. NCI-4877
1. Viral Biology Branch
OASDO, Etiology
2. Virus & Disease Modification
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Modification of normal and neoplastic cells by oncogenic and non oncogenic viruses

Previous Serial Number: Same

Principal Investigator: Dr. Wilna A. Woods

Other Investigators:
Dr. M. A. Chirigos, VBB, NCI (NCI-4875)
Dr. Willie Turner, VBB, NCI (NCI-4889)
Dr. John W. Pearson, VBB, NCI (NCI-4878)
Dr. Donald B. Schwartz, VBB, NCI (NCI-4879)
Dr. Gary Pearson, VBB, NCI (NCI-4880)
Dr. Harish C. Chopra, VBB, NCI (NCI-4874)
Dr. David Howell, VBB, NCI

Cooperating Units:

Inside NIH

Dr. Tom Cameron, CCNSC, NCI

Outside NIH

Dr. Roger Johnson, Ft. Detrick, Frederick, Md.
Dr. C. A. Bowles, Hazleton Laboratories
Falls Church, Va.
Dr. Bruce Wright, Hazleton Laboratories
Falls Church, Va.
Dr. William Kerber, Hazleton Laboratories
Falls Church, Va.
Dr. William Jurgelsky, NIEHS
Dr. G. Spahn, Microbiological Associates, Inc.
Walkersville, Maryland
Dr. T. Kawakami, University of California
Davis, California

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

(1) To investigate intracellular interactions between oncogenic and non-oncogenic viruses in vitro.
(2) To investigate alteration of antigenic composition of tumor cell membranes by infection with non-oncogenic viruses.
(3) To investigate use of tumor cells with altered membrane antigenic profiles in immunotherapy and prophylactic vaccination.
(4) To investigate the effect of chemotherapeutic agents on oncorna virus replication in vitro.

Methods Employed:

A. In vitro systems:
1. Cell lines of murine, canine, opossum, and human origin have been established and characterized. These lines can now be modified experimentally for basic immunologic and virologic studies.
2. ^{3}H-uridine incorporation into particles banding at 1.16 in a density gradient centrifugation is being used to identify presence of RNA in viral particles and demonstrate the presence of viral particles in cells.
3. Radio isotope procedures for detecting and characterizing RNA, DNA, and protein synthesis are now established on a routine basis.
4. Routine procedures used in this laboratory include virus titrations (focus formation by MSV-M, X-C, plaque formation by lytic virus, fluorescent focus for MuLV) and antigenic determinations (membrane fluorescence, cytotoxic antibody, fluorescent antibody staining of fixed cells, CF, hemagglutination inhibition, and neutralizing antibody titrations).
B. In vivo systems:
1. AKR and D_2AK mice are used for assay of AKR-A tumorigenicity and effectiveness of immunotherapy procedures.
2. CDF_1 mice are used for assay of the in vitro grown RCS tumor cell line and for immunotherapy experiments on this tumor.
3. BALB/c mice are used in MSV-M studies.
4. Beagle dogs are used for assay of immuno- and chemotherapy experiments at Hazleton Laboratories, Inc.

Major Findings:

1. Studies on the interaction of an oncogenic virus (MSV-M) and an arbovirus (Guaroa) in tissue culture have been completed and accepted for publication. Briefly, a cell line derived from a murine sarcoma virus (Moloney pseudotype)-induced tumor has been established. It retains oncogenicity, releases both sarcoma and leukemia viruses, and has virus-induced cellular antigens.
2. The characterization of the MSC cell line derived from a BALB/c tumor has been completed and the description published. The line is currently being used by several other investigators in this branch for immunological studies and was recently sent to Dr. Steven Russell of Scripps Research Institute, LaJolla, California.

3. Cross-protection studies using the MSV-M virus derived from the MSC cell line and MSV-M virus prepared from the original tumor homogenate are underway. The cell-line derived virus induces tumors which regress in only 20% of animals inoculated with 10^3 TD_{50} (median tumor dose). Survivors are protected against challenge with the MSC derived virus. When challenged with 10^3 TD_{50} of tumor homogenate virus, these regressor animals are protected. Conversely, when animals which have regressed tumors induced by the tumor homogenate virus are then challenged with MSC-derived virus, no protection can be demonstrated. These viruses are not distinguishable by conventional neutralization techniques, and the tumors are not antigenically distinguishable by fluorescent, or cytotoxic antibody or by immune lymphocytes. The lack of in vivo cross-protection can be due to (1) selection of a mutant virus in vitro, which induces a new TSTA on tumor cells or (2) mutation of the virus in vitro for new TSTA induction.
4. Work is in progress in collaboration with Dr. D. B. Schwartz using the MSC cell line to elucidate in vitro the immunological status of animals inoculated with MSVM and BCG. Levels of cytotoxic antibody, blocking antibody, neutralizing antibody as well as cellular immune ^{51}Cr release are being studied.
5. Characterization of cell lines derived from a reticulum cell sarcoma of CDF_1 mice and an apparently non-infectious "C-type" virus demonstrated in these cell lines has been completed and a manuscript is in preparation. Using discontinuous ficol gradient separation, two cellular types were isolated several times from the tumors of CDF_1 mice: (1) a round cell, with little adhesion to substrate, which produces typical tumors in CDF_1 mice, and (2) a fibroblastic cell, non-contact inhibited, with random orientation, which does not produce tumors when 10^6 - 10^8 are inoculated into CDF_1 mice. The parental in vivo passage tumor does not contain C-type particles, nor elicit an X-C reaction 1-2 days after seeding in culture. Both in vitro cell types produce C-type particles 8 days after in vitro cultivation and elicit XC syncytia formation. Attempts to infect NIH Swiss BALB/c, or CDF_1 embryo fibroblasts have been unsuccessful.

Immunization of CDF_1 animals with the virus carrying non-tumorigenic fibroblastic line was not successful, indicating that presence of the non-infectious virus will not protect against the tumor cell. This correlates with the demonstration of colony inhibition by lymphocytes from tumor-bearing animals against the "round" tumorigenic cells, but not against the fibroblastic, non tumorigenic cells.

Lindeman has reported protection of mice against Ehrlich ascites cells using vesicular stomatitis virus (VSV) infected cells and cell lysates. Both cells derived from the RCS tumor were found to be susceptible to lytic infection by vesicular stomatitis virus (VSV), with induction of VSV - viral related neoantigens on the cell surface. Therefore, immunization studies were initiated to determine if such infected cells or their lysates could immunize CDF_1 mice against challenge with RCS tumor cells. However, no protection was demonstrated, and indeed some enhancement was seen. Cytotoxic or membrane fluorescent antibodies could not be demonstrated, although VSV neutralizing antibodies were present in high titer. Experiments determining the blocking antibody level in

such animals and the effect of prior VSV infection on this antibody and on the immunological competence of the animals is planned.
6. Studies on virus infection of the continuous lymphoblastic cell line (AKRA) derived from a spontaneous AKR mouse tumor have been completed and a manuscript is in preparation. This cell line was shown to be very susceptible to chronic infection by a variety of viruses, with concomitant loss of Gross antigens. Simultaneous infection of all cells was achieved with one arbovirus, Germiston. Significantly, this virus was the only one to reduce tumorigenicity and elicit protection upon challenge with the virulent cell line. Further studies to elucidate the mechanism are planned.
7. Simmons et al have demonstrated alteration of surface antigens by neuraminidase treatment, and immunization with treated cells against tumor challenge with virulent tumor cells. We have treated AKRA cells with neuraminidase and have demonstrated some loss of Gross membrane fluorescent antigen with such treatment. To date, however, no protection has been demonstrable.
8. In collaboration with Dr. Willie Turner, we have studied production of V antigen in non viral producing mouse macrophages transformed by SV_{40} virus. Viral antigen was found in some cells which had been co-infected with MSVM.
9. Experiments are underway to determine the significance of the "C" type virus demonstrated by Armstrong et al in the continuous porcine kidney cell line PK-15. So far, we have demonstrated both nuclear and cytoplasmic-antigens (in about 25% of PK-15 cells) by fluorescent antibody techniques using serum from weanling pigs inoculated with concentrated purified virus derived from PK-15 cells.

Demonstration of infectivity for other porcine kidney cells is underway. The PK-15 cell and the virus derived from it has been shown to be negative for mouse and cat gs-1 antigen and for gs-3 antigen.

10. As part of a collaborative study with Dr. V. Berg and Dr. G. Pearson, the WFV embryo cell line was demonstrated to be negative for mouse and cat gs-1 and gs-3 antigen by fluorescent antibody techniques.
11. In cooperation with Dr. T. Cameron, NCI and Dr. William Kerber of Hazleton Laboratories, preliminary studies on chemo-immunotherapy of spontaneous canine lymphosarcoma have been completed. Dogs were treated with cytoxan or a combination of prednisone-cytoxan to obtain partial remission of tumor. The recovery of immune competence after treatment was tested by response to canine distemper vaccine and to monkey erythrocytes. One dog was treated with a continuous cell line derived from dog mast cell sarcoma infected with measles virus. The remission following this treatment was somewhat longer than previous remissions obtained with cytoxan alone. The significance of this finding awaits further suitable dogs and adaptation of in vitro immunologic tests to the canine system.
12. In cooperation with Dr. Charles Bowles of Hazleton Laboratories, incorporation of ^{3}H-uridine in in vitro cultivated chronically infected murine tumor cells has been investigated. Labeling of RNA banding at 1.16 in a sucrose gradient was more pronounced in cell lysate than in supernatant culture fluids. However, similar labeling was noted in normal mouse embryo fibroblasts. Characterization of the RNA of murine leukemia virus-producing cells and normal murine cells banding at 1.16 is underway.

Labeling of dog mast cell sarcoma cells (32043) with ^{3}H-uridine demonstrated a peak at 1.16, which could be passed to normal dog embryo fibroblast cells, which do not incorporate ^{3}H-uridine in the 1.16 fraction. The nature of this transmission is under investigation. Similarly, ^{3}H-uridine labeled RNA banding at 1.16 was isolated from a canine mammary tumor cell line. Several human sarcoma cell lines obtained from Dr. Adeline Hackett of Naval Biological Lab have been labeled with similar results.

13. In cooperation with Dr. William Jurgelsky of NIEHS, several tumors induced in opossums with chemical carcinogens have been established in vitro. Two tumors of developing tooth buds and 2 nephroblastomas have been established. The cultured cells have been inoculated into opossums of various ages for tumorigenicity studies, and have been submitted for electron microscopic analysis.

14. The mechanism of action of streptonigrin, a potential chemotherapeutic drug, is being investigated in collaboration with Dr. G. Spahn of Microbiological Associates.

In concentrations as low as 0.5 ng/culture, RNA synthesis of mouse embryo fibroblasts is inhibited within 30 minutes. Protein synthesis is not inhibited; DNA synthesis is inhibited only after 24 hours exposure and 5 ng/culture. The effect on cell growth in mass culture and by cloning efficiency has also been determined. In cultures initiated with 10^{4} cells, as little as 10 ng caused a 50% inhibition in growth at 48 hours. Cloning efficiency (100 cells/dish seeded) was inhibited 90% by as little as 1.0 ng/100 cells.

The effect of the drug on chronically infected MSC cells, derived from a MSVM induced mouse sarcoma, is also being investigated. Preliminary results indicate that the drug does not inhibit RNA synthesis in these cells at non-toxic levels. Cell growth is inhibited by 5 ng/10^{5} cells, and cloning efficiency is 50% inhibited by 1.0 ng/10^{2} cells. Viral replication does not appear to be inhibited, in contrast to findings with primary infection of MEF cells. Cell clones are now being isolated in the presence of streptonigrin and the antigenic and virological characteristics will be determined.

The effect of the drug on non-oncogenic viruses is also being investigated. Preliminary results for New Castle Disease Virus replicating in Vero cells indicates transient inhibition of cellular RNA and a slightly prolonged replicative cycle.

Significance to Biomedical Research and the Program of the Institute:

The study of oncogenic virus, non-oncogenic virus interaction is of possible importance to determining the "trigger" mechanism for tumor induction in animals (including man) carrying the oncogenic virus potential from conception. The cause-effect relationship between the oncorna viruses and tumors and the fundamental cellular changes leading to oncogenicity may also be elucidated by detailed biochemical studies of the 2 cell lines isolated from the reticulum cell sarcoma.

The study of mechanisms of action of potentially therapeutic drugs on both cell and virus replication has potential significance for screening such agents in vitro before application in vivo.

The study of ^{3}H-uridine incorporation into high molecular weight RNA of malignant cells will be of importance in detecting such cells bearing defective viral genomes. The interaction of oncogenic and non-oncogenic viruses should also be determined to insure complete safety in such cases.

Proposed Course:

1. The significance of ^{3}H-uridine incorporation into high molecular weight RNA of malignant cells of man and other animals will be determined.
2. The effectiveness of streptonigrin for prevention and interruption of viral and cellular replication will be pursued.
3. Virus-virus interactions involving Gross leukemia virus and Germiston arbovirus, as well as, MSV and Guaroa arbovirus will be investigated.
4. The significance of oncorna virus producing non-malignant cells and malignant cells of the RCS system will be determined.

Honors and Awards:

None

Publications:

Massicot, J. G., Woods, W. A., and Chirigos, M. A.: Cell line derived from a murine sarcoma virus (Moloney Pseudotype)-Induced tumor: Cultural, Antigenic, and Virological Properties. Appl. Micro. 22: No. 6, p. 1119-1122. December 1971.

Woods, W. A., Turner, W., and Chirigos, M. A.: Co-Infection of mouse spleen cells with murine sarcoma virus and guaroa virus. Appl. Micro. (In Press).

Bowles, C. A., Kerber, W. T., Rangan, S. R. S., Woods, W. A., and Jensen, E. M.: Studies of a transplantable canine tumor. Vth International Symposium on Comparative Leukemia Research. Padova-Venice (In Press).

Serial No. NCI-4878
1. Viral Biology Branch
OASDVO, Etiology
2. Virus & Disease Modification
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Modification of Oncogenic Viruses and Disease Induction by Immunological, Biological, Chemical and Physical Methods

Previous Serial Number: Same

Principle Investigator: Dr. John W. Pearson

Other Investigators:
Dr. M. A. Chirigos, VBB, NCI (NCI-4875)
Dr. Gary Pearson VBB, NCI
Dr. Donald Schwartz, VBB, NCI (NCI-4879)
Dr. Wilna A. Woods, VBB, NCI (NCI-4877)

Cooperating Units:

Inside NIH

Dr. Sotiros Chaparas, LBP, DBS
Dr. Jerry Rice, BB, NCI
Dr. Holger Kirschner, D, LM

Outside NIH

Dr. G. Spahn, Microbiological Associates, Inc.
Bethesda, Maryland

Man Years:

Total:	3.0
Professional:	1.0
Other:	2.0

Project Description

Objectives:

The overall aim of this project is to control virus induced, spontaneous and transplantable leukemias and lymphomas by several therapeutic approaches. The therapeutic approaches encompass individual or combination drug therapy, immunotherapeutic methods, vaccines, and the application of interferon. Any therapy leading to effective control will be submitted for pre-clinical testing, evaluation and eventual clinical trial.

Methods Employed:

Mouse Virus and Tumor Animal Systems:
1. The Friend, Rauscher, Moloney leukemogenic viruses, the Moloney sarcoma virus, and the plasma passage virus variant (MSV-PV) are employed for in vivo studies.
2. A Moloney lymphoid leukemia ascites line (LSTRA), a Graffi leukemia virus transplantable tumor line, a transplantable tumor line induced by Gross virus and a sarcoma transplantable tumor line of MSV(PV) are employed for in vivo studies in mice.
3. Studies in AKR mice which develop spontaneous leukemia at 7-9 months of age.
4. Rat transplantable lines are now under investigation and are being carried not only in vivo but also in vitro: (a) The Dunning lymphosarcoma transplantable line; (b) a transplantable leukemia induced by the Gross virus; (c) a sarcoma transplantable tumor line induced by MSV(M) virus and; (d) a Nova transplantable leukemia.

Major Findings:

1. Chemo-Immunostimulation Therapy: Investigations have been conducted with a murine lymphoid leukemia (LSTRA) where immunostimulators have been used in combination with drug therapy in order to achieve complete remission in diseased animals. Subcutaneous inoculation of 1×10^4 ascites cells, repeatedly and reproducibly, result in tumor death within 12-15 days. Drug treatment (BCNU) consistently results in a doubling in survival time and a 12-15% long term survival. The period of remission before relapse was established by bioassay techniques. A remission period of 6-8 days following drug treatment was established where no systemic disease could be detected prior to relapse. Employing two non-specific immune stimulators, BCG (Glaxo strain) and Corynebacterium granulosum (CG) immediately following drug induced remission or prior to relapse, we observed a substantial increase in survival time and percentage of survivors. This response has been consistently reproduced with 70-80% survivors (disease free) as compared to 12-15% survivors obtained in groups treated only with the drug. Similar studies are in progress in a rat leukemia system and in dogs. Investigations are in progress to determine the mechanism(s) of action of both BCG and CG.

Immunostimulation studies used in combination with drug therapy are currently underway against the spontaneous leukemia in AKR mice. Treatment of diseased animals with 5 azacytidine, BCNU and prednisone has resulted in remission (absence of splenomegaly and/or lymphadenopathy) for approximately 10 days. Once the best regimen of treatment is defined, BCG and CG will be given during the period of remission in order to determine whether the period of remission will be extended and/or whether long term survival without disease will be attained.

Subcutaneous inoculation of approximately 2×10^4 Dunning tumor cells results in the death of all animals within 18 to 29 days. Complete tumor regression was observed within 6 days when tumor bearing animals were treated with 45 mg/kg of cytoxan. Tumor reappeared at the original site of inoculation after

approximately 12 days. When BCG and CG were administered at a time when the drug treated animals were in a state of remission, a high number of long term survivors were obtained, i.e. BCG (66%) and CG (50%). Interestingly, no difference was observed as to the best time to administer the stimulators during the period of remission. Further studies with this system are in progress.

2. Chemo-Adoptive Therapy: Concurrently, studies have been underway to see what effect immune and normal spleen cells would have on extending the period of remission and/or increasing the number of long term survivors following drug therapy. LSTRA infected mice were treated on day 7 with either 25 or 20 mg/kg of BCNU in order to induce a state of remission. Animals were then treated with either single or multiple injections of immune or normal lymphoid cells. Interestingly, both immune and normal spleen cells, regardless of the number of treatments, yielded a high number of long term survivors (75-100%) following treatment with 25 mg/kg of BCNU. Why normal spleen cells were as effective as immune lymphocytes is not known at this time; however, this phenomenon is currently under investigation. In contrast, immune and normal spleen cells when administered to BCNU treated animals (20 mg/kg) failed to prolong host survival beyond that observed with BCNU therapy alone (MST = 28.0 days). It was obvious from this study that a reduced tumor load was essential in order for lymphocytes to exert an immunotherapeutic effect on the host. Similarly, the use of sensitized spleen cells alone failed to control the established disease.

3. Chemo-Interferon Therapy: Studies were undertaken to evaluate the effect of pI:C and pI:C/p-D-lysine when administered to animals in a state of remission following drug therapy. LSTRA infected mice were treated with 25 mg/kg of BCNU on day 7, a time when the disease is systemic. The results showed that 100 µg's of pI:C or pI:C/p-D-lysine given 5 days after drug therapy, a time when animals were in remission, and treated weekly thereafter for 3 weeks yielded 60% long term survivors. In contrast, only 33% of the animals survived in the group treated only with drug. Similarly, where the stimulators (50 µg's) were given twice daily for a week (day 12 to day 16) following drug therapy on day 7, 65% long term survivors were obtained. Investigations are underway to define the mechanism of action as both pI:C and pI:C/p-D-lysine. Both are known to be good interferon stimulators and immunoenhancers.

Significance to Biomedical Research and the Program of the Institute:

These studies are directed toward the prevention, treatment and control of virus-induced or spontaneous leukemias, lymphomas, and sarcomas in animal systems. The use of drugs, either alone or in combination with immunostimulators, immunotherapy and vaccines may elucidate factors improving the effectiveness of therapeutic measures.

Proposed Course:

Research in each of the areas described under objectives will continue.

More emphasis will be placed in maintaining prolonged periods of remission following chemotherapy. The use of immunostimulators alone and in combination with syngeneic and allogeneic vaccines will be investigated in both mouse and rat leukemia and sarcomas following drug therapy. Similar approaches will be attempted against the spontaneous AKR leukemia. It is hoped that information gained from these investigations may be applied toward the control of human cancer.

Honors and Awards:

None

Supply of Transplantable Tumor and Leukemia Cell Lines:

Over the past year several transplantable lines have been sent to the following investigators at their request: (a) Dr. Werner Braun, Rutgers, The State University; (b) Dr. Ronald Herbermann, NCI; (c) Dr. Mark Lippman, NCI.

Publications:

Pearson, J. W., Pearson, G. R., Gibson, W. T., Chermann, J. C. and Chirigos, M. A.: Combined chemo-immunostimulation therapy against murine leukemia. Cancer Research. (In Press).

Turner, W., Ebert, P., Riechers, L., Pearson, J. W. and Chirigos, M. A.: Elucidation of the nature of the murine oncogenic virus inhibitor isolated from JLS-V5 cell line (36044). Proc. Soc. Exp. Biol. and Med. 138: 1030-1034, 1971.

Chirigos, M. A., Pearson, J. W., Spahn, G. and Rutman, R.: Current studies on oncorna virus therapy. Vth International Symposium on Comparative Leukemia Research. Padova-Venice (In Press).

Serial No. NCI-4879
1. Viral Biology Branch
OASDO, Etiology
2. Virus & Disease Modification Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Attempts to Enhance Cellular Immunity with BCG

Previous Serial Number: Same

Principal Investigator: Dr. Donald B. Schwartz

Other Investigators: None

Cooperating Units: Dr. Michael A. Chirigos (NCI-4875)
Dr. Wilna Woods (NCI-4877)
Dr. Jack Pearson (NCI-4878)

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

1. To study the mechanisms involved in the inhibition of murine sarcoma virus oncogenesis by BCG.

2. To study various preparations of BCG by demonstrating in comparative trials of different BCG's a variation in ability to inhibit viral oncogenesis.

3. To extend the findings in these studies to other animal systems.

Methods Employed:

1. In Vivo : (a) It has been established that intramuscular injection of murine sarcoma virus (MSV) into mice causes large tumors. The development of these tumors can be inhibited if BCG (an attenuated strain of Mycobacterium bovis) is inoculated along with the virus, and the addition of previous immunization of these mice with BCG 28 days before inoculation of the mice with the mixture of MSV and BCG is associated with the most impressive inhibition of oncogenesis. (b) Dr. Jack Pearson has shown that survival of CDF_1 mice with chemotherapeutically treated LSTRA tumors can be prolonged if immuno-stimulation follows chemotherapy.

2. In Vitro: (a) Tumor cells can be killed by immune lymphoctyes. Previously labelled tumor cells release their label when killed by immune lymphocytes. As assay to determine the amount of radioactive label released by the tumor cells enables us to quantitate the effectiveness of immune lymphocytes. (b) Serum factors that may enhance tumor growth can be detected by determining their ability to inhibit the killing of tumor cells (in the above radio-label assay) by lymphocytes. This work is now in progress. (c) Tumor cells can be killed by antibody, and an assay detecting the release of radioactivity from labelled tumor cells can be used to quantitate the amount of cytotoxic antibody present in sera of mice with tumors. (d) Antibodies capable of neutralizing viruses can be detected by incubating the antibody with the virus before doing quantitative focus forming assays for virus. This work is now in progress. (e) Immune lymphocytes can be transformed by various means and the amount of transformation quantitated by the uptake of radioactive thymidine by the lymphocytes. This work is now in progress. (f) The incorporation of thymidine by target cells can be inhibited by immune lymphocytes. The amount of inhibition can be quantitated by measuring the amount of thymidine incorporation.

Major Findings:

1. In Vivo: (a) Inhibition of MSV oncogenesis: The results of our in vivo findings have been published recently (see list of publications). To summarize, viral oncogenesis can be inhibited by the simultaneous inoculation of BCG. Unpublished information reveals that contact of the BCG with the virus is required. Mice inoculated with MSV in one leg and inoculated simultaneously with BCG in the contralateral leg actually develop larger tumors (enhancement) associated with shortened survival when compared to mice inoculated with MSV alone.

The ability to inhibit MSV oncogenesis by BCG has enabled us to evaluate different preparations of BCG. Preliminary findings indicate that there are no differences among strains of BCG in ability to inhibit oncogenesis; however, variations in the present techniques of producing BCG vaccines do result in differences in ability to inhibit oncogenesis. (b) Immune stimulation of chemotherapeutically treated LSTRA mouse tumors: Using the system described originally by Dr. Jack Pearson we have been able to demonstrate unusual dose-time relationships necessary for most effective immuno-stimulation. Larger doses of immune stimulants (BCG and Corynebacterium) are required for prolonging survival when used shortly after chemotherapy (early immunotherapy) than when used several days later (delayed immunotherapy). The smaller doses of immune stimulants effective with delayed immunotherapy are not effective with early immunotherapy; and of greatest interest, the large doses effective in early immunotherapy are not effective when used for delayed therapy.

2. In Vitro: (a) Mice with MSV-M induced tumors have lymphocytes capable of killing labelled tumor cells in vitro as demonstrated by the release of CR-51 (unpublished new data). Mice without tumors, because induction of tumors by MSV-M was inhibited by the addition of simultaneous inoculation of BCG, also have lymphocytes capable of killing tumor cells, but their lymphocytes are

not as effective as lymphocytes from mice with tumors (unpublished data). Lymphocytes from mice treated with BCG alone possess no activity and respond similar to normal lymphocytes. (b) Attempts to transform lymphocytes with tumor cells as the stimulating antigen: We are still working on the demonstration of cellular immunity using lymphocyte transformation techniques. (c) Cytotoxic antibodies can not be demonstrated in the murine sarcoma virus inoculated animal either because 1) they do not exist, 2) the target cells are not as sensitive as in vivo cells, 3) the incubation period was not long enough (unpublished data). We have shown that sera from rats immune to MSV-M is cytotoxic for the target tumor cells.

Significance to Biomedical Research and the National Institutes of Health Program

Studying the effect of immuno-therapy in animal systems can give us further insight into the mechanisms responsible for immuno-therapeutic success. We can also learn ways to best manipulate immuno-therapy for maximal results and apply the information gained in human applications. The results of our soon to be reported observations on the variations in BCG preparations may have a profound interest for people using BCG in treating human malignancies. Initial hypothesis on our observations of the unique dose-time relationship necessary for optimal benefit from immunotherapy following chemotherapy may totally change previous dogma stating that immunotherapy is most effective in a host whose tumor has been completely suppressed with chemotherapy.

Honors and Awards:

None

Publications:

Schwartz, D. B., Zbar, B., Gibson, W. T. and Chirigos, M. A.: Inhibition of Murine Sarcoma Virus Oncogenesis with Living BCG. Int. J. Cancer 8: 320-325, 1971.

Serial No. NCI-4880
1. Viral Biology Branch
OASDVO, Etiology
2. Experimental Pathology
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Immunological and Virological Studies on Human and Animal Tumors

Previous Serial Number: Same

Principal Investigator: Dr. Gary Pearson

Other Investigators: Inside NIH
Dr. Jack Pearson, Viral Biology Branch, NCI (NCI-4878)
Dr. Paul Ebert, Viral Biology Branch, NCI (NCI-4876)
Dr. Harish Chopra, Viral Biology Branch, NCI (NCI-4874)
Dr. Dharam Ablashi, Viral Leukemia and Lymphoma Branch, NCI (NCI-4834)
Dr. Paul Levine, Viral Leukemia and Lymphoma Branch, NCI (NCI-4822)

Outside NIH:
Dr. Victor Bergs, University of Miami, Miami, Florida
Dr. Harvey Rabin, Bionetics Research Laboratories, Kensington, Maryland

Man Years:

Total:	3.5
Professional:	1.0
Other:	2.5

Project Description

Objectives:

1. To investigate the effectiveness of different immunological measures in the control of tumor induction and development.

2. To investigate the susceptibility of non-human primates to infection with Epstein-Barr virus.

3. To characterize host humoral and cellular immune responses to selected oncogenic viruses.

4. Collaboration with other scientists at NCI.

Methods Employed:

1. Mice were immunized with MSV or cells producing MSV. At appropriate times following the immunizations, these mice were bled and the sera pooled into different groups depending on the donor mice treatment. These sera were heat-inactivated and then tested for antibodies directed against MSV-associated antigens by membrane immunofluorescence, neutralization of the X-C reaction and cytotoxicity. Recipient mice were then inoculated intraperitoneally with 0.2 ml of a test serum. Three or four days later the mice were challenged intramuscularly with known members of syngenic tumor cells. These animals were palpated twice weekly for the appearance of tumors.

2. EBV concentrates were prepared from culture medium taken from the HR1K line of lymphoblastoid cells. These culture fluids were clarified by low speed centrifugation, and the virus was then pelletized by centrifugation at 2700 G for 90 minutes. The virus pellet was resuspended in 1640 + 10% fetal calf serum at a volume equivalent to 1/500 - 1/1000 concentration of the original volume and filtered through a .8w filter. The infectivity of the virus concentrates for Raji cells was determined by the anti-EA assay of the Henles'. Only virus preparations containing at least 10^3 infectious units were used for *in vitro* infection of primate lymphoid cells.

Primate bone marrow cells were exposed to infectious EBV for 1 hour at 37°C. Following this incubation period, the cells were resuspended in 1640 + 20% fetal calf serum and incubated at 37°C. The cultures were followed weekly for morphological signs of transformation.

3. Adult W/Fu rats were inoculated subcutaneously with the virus-producing WF-1 cell line (W/Fu origin). When the tumors were 20-25 mm in size, the rats were bled by cardiac puncture and the serum fractions collected. These sera were heat-inactivated and tested for antibodies directed against virus-associated membrane antigens by membrane fluorescence.

Cell-mediated immunity tests were performed using the chromium^{51} cytotoxic assay previously described (Pearson, *et al*, 1970).

Briefly, target cells were labeled with chromium^{51} and then added to screw-cap glass vials. Spleen cells obtained from normal donors or rats immunized with irradiated tumor cells were then added to the vials at 100-200:1 lymphoid: target cell ratio. The mixtures were then incubated for 18-24 hours at 37°C. Following this incubation period, the supernatants were collected, clarified by centrifugation and the radioactivity in the cell-free supernatants counted in a Packard scintillation counter.

Major Findings:

1. Studies were initiated to investigate the effectiveness of immune serum in preventing the outgrowth of Moloney sarcoma virus-induced tumor cells. Experiments were designed to include sera previously reported by the Hellstroms to contain blocking or de-blocking factors. Serum donors included (1) Balb/C mice bearing progressively growing MSV-induced tumors (referred to as progressor serum); (2) Balb/C mice in which the MSV-induced tumors regressed (regressor serum); and (4) normal Balb/C or CDF_1 serum. These sera were titrated by membrane fluorescence, neutralization (X-C test), and cytotoxicity to determine if antibodies detected by any of these methods would relate to the effectiveness of the sera in vivo.

Tumor growth was inhibited in mice preinoculated with the regressor or allogenic serum. In contrast, tumor growth was enhanced in mice pretreated with the progressor serum.

These sera were also tested for effectiveness against a highly lethal Moloney virus-induced leukemia (LSTRA). Only the allogenic serum inhibited tumor production.

Serological analysis of these sera revealed the following titers:

	Test		
Serum	MF	Neut.	Cyto.
progressor	<10	<10	neg.
regressor	64	53	neg.
allogenic	≥128	227	neg.

These results indicated that the effectiveness of a serum in inhibiting the growth of Moloney virus-induced tumor cells could be measured by its anti-membrane and neutralizing titers as well as by the previously reported (Hellstroms) blocking factors.

We are now in the process of testing the effectiveness of these sera in diseased animals before and after treatment with chemotherapy.

Studies are now underway attempting to determine the active factor in the protective sera. It is intriguing that these sera were effective even when administered as late as 3-4 days before challenge. The half-life of mouse gamma globulin is 2-5 days.

This suggests that some factor other than antibody, such as a soluble transfer factor, might be responsible for the transfer of immunity. Along these lines, it is interesting to note that 50% of the serum-treated mice

Studies were also undertaken to investigate the cellular immune response to WF-1 tumor cells. These experiments utilized the chromium^{51} release cytotoxic test previously described (Pearson, et al, 1970) to monitor lymphocyte cytoxicity in vitro.

Spleen cells from adult W/Fu rats immunized with multiple injections of irradiated WF-1 tumor cells were tested against a variety of cell lines for specific cytotoxic activity. Cytotoxic effects were demonstrated against the WF-1, R-35 and normal W/Fu embryo cell lines. Spleens from rats bearing WF-1 tumors were ineffective as were spleens from rats immunized with BCG. These results indicated that anti-WF-1 cytotoxic spleen cells were sensitized by an antigen shared with normal embryonic cells. To test this further, adult W/Fu rats were immunized with normal embryonic cells or adult spleens. Lymphoid cells from rats immunized with normal embryonic cells but not with adult spleen cells were cytotoxic to the same spectrum of cells previously noted with anti-WF-1 spleen cells. These results indicated that (1) embryonic antigens were expressed in the membranes of different tumor lines and (2) that these antigens could provoke an immune response in appropriately immunized adult animals. Further investigations are now in progress to better define the specificity of this cytotoxicity and to determine the relationship of embryonic antigens to tumor-specific transplantation antigens.

4. Approximately 12 different cell lines are now being carried in this laboratory. They have been provided to other investigators both inside and outside NIH on their request for a variety of virological and biochemical investigations.

Significance to Biomedical Research and the Program of the Institute:

1. Investigations on the possible use of immunological factors for controlling tumor growth are of obvious importance to the Program. Recent evidence from the Hellstrom Laboratory indicates that sera containing de-blocking factors can induce tumor regressions. It is important to determine not only what is the nature of the active factor in these sera but also what other tests can be utilized for selecting sera for potential use in therapy.

2. Indirect evidence has accumulated associating EB virus with Burkitt's lymphoma. However, direct evidence is lacking. It is important to attempt to establish EB viral infection in an animal model so that experimental investigations can be undertaken to determine the relationship between EB virus and oncogenesis in man.

3. A number of rat type C virus particles associated with rat malignancies have been isolated. The etiological significance of these particles however is still unknown. Immunological investigations in the relationship of these viruses to each other as well as to oncogenic viruses in other species could provide useful information for determining their role in the

that initially rejected a challenge dose of MSV-induced tumor cells rejected a second and larger challenge inoculum administered 45 days later. One would not have expected this if the passively administered antibody was solely responsible for the initial protection. We cannot eliminate the possibility that these mice were actively sensitized by the initial challenge, but our preliminary data indicates that this may not be the case. Further studies are needed to clarify these points and to define the factors responsible for transferring protection.

2. Progress in experiments designed to investigate the susceptibility of nonhuman primates to infectious EBV concentrates was hampered by the unavailability of New World monkeys. Recently, however, bone marrow specimens from 1 marmoset and 1 owl monkey were provided by Bionetics Research Laboratories for in vitro studies. These cells were exposed to EBV concentrates of known infectivity as determined by the anti-EA assay described by the Henles. Following exposure to the virus, the cells were monitored for EBV antigen production and for signs of transformation. EBV antigens were not detected in these cultures over a 1 month observation period nor were there any indications that the cells were transformed.

3. Rat cell lines producing rat type C virus particles were investigated for the presence of virus-associated membrane antigens similar to those produced by other type C virus particles. The 3 cell lines investigated were: (1) WF-1 cell line established by V. Bergs from normal W/Fu rat embryo cells; (2) RMTL-8 cell line established by V. Bergs from a chemically-induced mammary tumor in a Sprague-Dawley rat; and (3) R-35 cell line established from a spontaneous mammary carcinoma of a Sprague-Dawley rat. All 3 cell lines were examined by electron microscopy and found to be producing rat type C virus particles.

Sera from W/Fu rats bearing WF-1 tumors contained antibody that reacted with WF-1 cells by membrane fluorescence but not with normal W/Fu embryo cells. Absorption experiments confirmed the specificity of this reaction.

These results suggested that the antibodies detected by membrane fluorescence were directed against viral associated antigens on the surface of WF-1 cells. Interestingly, some of these sera also reacted with the two other virus-positive cell lines (RMTL-8, R-35), but not with virus negative cell lines, including normal Sprague-Dawley mammary cells. Absorption experiments verified that the R-35 and RMTL-8 cell lines contained membrane antigens cross-reacting with the WF-1 cells. These results suggested that the type C virus particles found in cell lines originating from different tissues as well as from different strains of rats were antigenically closely related if not identical. Preliminary neutralization experiments provided further evidence for this relationship.

Both the RMTL-8 virus (referred to as BV-1) and the WF-1 virus produce lesions in REL cell cultures. Antiserum prepared against BV-1 virus neutralized the infectivity of both of these viruses.

induction of rat tumors. It is also of interest to clarify the relationship of embryonic antigens to tumor-specific transplantation antigens.

Proposed Course:

1. Studies on the effectiveness of well-characterized immune serum in transferring immunity to tumor transplants will be continued. Efforts will concentrate on determining the effect of sera when inoculated into diseased animals. Experiments will also be undertaken to define the active factors in these sera.

2. Attempts to establish a model system in nonhuman primates for investigating EBV infections will be continued as animals become available. Monkeys will be inoculated with highly infectious EBV concentrates by different routes and then monitored for evidence of infection by a number of different parameters. These will include (a) immunological responses, (b) blood counts, (c) virus rescue studies and (d) a variety of tissue culture assays. Efforts to infect lymphoid cells in vitro with EBV concentrates will also be continued.

3. Immunological and virological aspects of herpes virus saimiri infections will be initiated. These will include (a) definition of the different antibodies produced following HSV infection and the effects of tumor growth on the levels of these antibodies; (b) studies on the cellular immune response and attempts to define the role this response plays in the pathogenesis of ASV-induced disease; (c) determination of the effectiveness of serum containing high levels of anti-membrane antigen antibodies in the control of ASV infections; and (d) studies on the possible interaction of HSV with type C virus particles.

4. Immunological studies investigating the relationship of different rat type C virus isolates will be continued. The relationship of embryonic antigen to tumor-specific transplantation antigens will also be investigated.

Other Activities:

Dr. Pearson is Executive Secretary of the Immunology-Epidemiology Segment, Project Officer on 7 contracts and Assistant Project Officer on 4 contracts within the Special Virus Cancer Program. He also is a member of the Resources and Logistics Advisory Committee.

Honors and Awards:

Dr. Pearson presented a seminar entitled "Immunological Investigations on Epstein-Barr Virus" at George Washington University in November 1971.

Publications:

None.

Serial No. NCI-4881
1. Viral Biology Branch
OASDVO, Etiology
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies with Epstein-Barr Virus (EBV). Host-Virus-Cell Relationships.

Previous Serial Number: Same

Principal Investigator: Dr. Virginia C. Dunkel

Other Investigators: Dr. Sam Margolis (NCI-4882)

Cooperating Units: None

Man Years:

Total:	3.8
Professional:	0.8
Other:	3.0

Project Description

Objectives:

(A) To study the nature of soluble antigens derived from EBV-infected and EBV-free lines and primary Burkitt lymphoma tissue.

(B) To determine the antibody status of primates to EBV antigens and whether sero-negative animals represent a suitable experimental animal for EBV studies.

Methods Employed:

The following methods are being applied to these studies: Immunofluorescence, complement fixation, micro immunodiffusion, immunoelectrophoresis, disc electrophoresis, column chromatography, and infectivity tests. Soluble antigens are prepared from large batches of tissue cultures and primary tumor specimens.

Major Findings:

A study on the antibody status of primates was completed. Sera from seven species of Old World monkeys, two species of New World monkeys and one specie of Prosimian were tested by indirect immunofluorescence tests for antibodies to EBV and EBV-related antigens. With acetone-fixed P3HR-1 and EB-3 cells, no fluorescence was observed with sera from the New World monkeys or Prosimian. Positive reactions were observed with all species of Old World monkeys tested, but differences in reactivity were found when the same sera were tested with either anti-monkey gamma globulin or anti-human IgG conjugates. In general there were more positive reactions and higher titers with the monkey sera and the anti-monkey gamma globulin conjugate than with the same sera and the anti-human IgG conjugate. In parallel tests with human sera and these two conjugates, some differences in titer were observed when P3HR-1 cells were used, but not with EB-3 cells. It thus appears that Old World monkeys may carry a herpes virus which is serologically similar to EBV or that they may be susceptible to infection with human EBV.

Further studies associated with primates led to the establishment of a lymphoblastoid cell line from the peripheral leukocytes of a rhesus monkey with a chemically induced myelogenous leukemic. The sera of the donor monkey contained no antibodies directed against EBV or EBV-related antigens. In addition EB virus could not be detected in the cells by immunofluorescence tests by electron microscopic observation. The cells are free of mycoplasma, do not contain SV40 V or T antigens, and do not harbor B-virus. They are also resistant to infection with EBV itself. The cells have been inoculated intracerebrally into newborn rhesus monkeys but to date there is no evidence of proliferation of the cells in these animals.

Studies were continued on the nature of the soluble antigens in human lymphoblastoid cell lines. Specific cellular fractions were prepared from two EBV-producing lymphoblastoid cell lines (P3J and P3HR-1) and two non-producing lines (Raji and NC-37) and tested for the presence of soluble antigens by immunodiffusion and complement fixation. Soluble antigen(s) was detected in the nuclear fraction of all cell lines, but only those cells producing EBV gave positive results by immunodiffusion. This antigen corresponded to the B antigen found in whole cell extracts of EBV-producing cells. In addition the "metochondrial" fraction and cell membrane fractions also contained soluble antigens. These antigens were distinct from the antigen found in the nucleus and did not correspond to any of those found in whole cell extracts. The exact relationship between these antigens and Epstein-Barr virus is yet to be determined.

Significance to Biomedical Research and the Program of the Institute:

The ability of an experimental primate sensitive to infection by EB would be extremely helpful in determining the oncogenic properties of EB virus.

The work done shows that Old World monkeys are susceptible to EBV or a virus very similar to the EB virus infecting man. It is possible that the New World monkeys may provide a working animal model system for EB virus since they are apparently devoid of infection with a very similar virus.

Proposed Course:

These studies will be terminated with the departure of the principal investigator.

Other Activities:

Project Officer on four and Assistant Project Officer on two research contracts within the Special Virus Cancer Program.

Honors and Awards:

None.

Publications:

Dunkel, V.C., and Myers, S.L.: Continuous lymphoblastoid cell line from a rhesus monkey with myelogenous leukemia. J. Nat. Cancer Inst. (In press).

Serial No. NCI-4882
1. Viral Biology Branch
   OASDVO, Etiology
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Investigations on the Biochemical Composition and Growth Kinetics of Mammalian Tumor Viruses.

Previous Serial Number: Same

Principal Investigator: Dr. S. Margolis

Other Investigators: Dr. V. C. Dunkel (NCI-4881)
Dr. H. C. Chopra (NCI-4874)

Cooperating Units: Inside NIH
None

Outside NIH
Bionetics Laboratories, Dr. D. Fabrizzio

Man Years:

Total:	1.9
Professional:	1.0
Other:	0.9

Project Description

Objectives:

To study the action of Mason-Pfizer monkey (M-PMV) virus on the growth rate, chromosomal distribution and RNA synthesis of chronically infected monkey embryo cells.

Methods Employed:

The following methods are being used in these studies: Tissue culture techniques, indirect immunofluorescence, chromosome enumeration techniques, monkey foreskin assay of M-PMV, RNA extraction, density gradient fractionation, polyacrylamide gel electrophoresis and radioactive tracer techniques.

Major Findings:

The molecular size of M-PMV is similar to that of other RNA tumor viruses (60-70S). In a chronically-infected cell system, virus production decreased to very low levels after 25-30 subcultures. The cells doubled

in saturation density and polyploidy in the culture dropped from an original 82 percent to 28 percent. This sequence of events was verified by infecting monkey embryo cells. From an initial 78 percent polyploidy, the number of polyploid cells was reduced to 30 percent at the ninth subculture. Experiments are in progress to determine whether there is a correlation between polyploidy and the rate of virus production.

The reduction of virus production observed over time in cells chronically infected with M-PMV may be due to a repression of virion maturation or of the production of viral RNA. After exposure of these cells to tritiated uridine for 24 hours, an increase in the labeling of 28S RNA in the absence of a significant increase in the total amount of spectrophotometrically measurable 28S RNA was observed. Attempts to separate a component similar in molecular size to ribosomal RNA was unsuccessful. However, the short term pulse and pulse chase labeling technique permitted assessment of the rate of synthesis of ribosomal RNA. The 32S and 28S RNA species were synthesized more rapidly in infected cells suggesting that the 28S RNA was newly synthesized. In addition, high molecular weight RNA turnover was more rapid in infected cells. These changes were observed in cells in fresh medium, which stimulates virus production. It is felt that the increase in RNA synthesis observed following virus infection of the cells is related to the synthesis of viral RNA, viral mRNA, or possibly a special 28S RNA required for synthesis of viral protein.

Significance to Biomedical Research and the Program of the Institute:

These studies were undertaken to contribute to the elucidation of two of the major problems in viral oncology: the nature of the event that prevents the formation of mature virus in many mammalian tumors and the intracellular events in the growth and maturation of oncorna virus.

Proposed Course:

These studies will be terminated.

Honors and Awards: None

Publications:

Margolis, S. A., Oie, H., Levy, H. B.: The effect of interferon, interferon inducers, or interferon induced viral resistance on subsequent interferon production. J. Gen. Virol. (In press)

Serial No. NCI-4883
1. Viral Biology Branch
OASDVO, Etiology
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 - June 30, 1972

Project Title: Immunity to Virus-Induced Tumors

Previous Serial Number: Same

Principal Investigator: Dr. Charles W. Boone

Other Investigators: Mr. Robert Koegel

Cooperating Units: None

Man Years:

Total:	3.5
Professional:	0.5
Other:	3.0

Project Description

Objectives:

1. To determine the mechanism of the cell-mediated immune response to virus-induced, chemically-induced and spontaneous tumors.

2. To evaluate various methods of immunotherapy in animal model systems.

3. To develop better assays of potential application to humans for the cellular and humoral immune response to cancer.

4. To characterize the structure and physiological behavior of tumor cell surface antigens.

Methods Employed:

1. Tumor-graft rejection assays for TSTA in mice.

2. Delayed hypersensitivity assays for tumor-associated antigens in mice.

3. Chromatographic and biochemical methods for making hybrid antibodies.

4. Application of the method of immune cell reconstitution of thymectomized and x-irradiated mice.

5. Application of isotopic methods to labeled cells and antibodies.

Major Findings:

1. Cell-free homogenates of influenza virus-infected SV40 transformed tumor cells induced tumor immunity whereas non-influenza virus-infected cell homogenates did not. This finding was extended to the more significant case of methylcholanthrene-induced fibrosarcomas. The influenza virus mediated adjuvant effect could not be produced by a mixture of egg grown virus and tumor homogenates or by neuraminidase treatment of cells prior to homogenization. Homogenates of virus-infected cells induced a delayed hypersensitivity reaction in tumor-immune but not normal mice.

2. The binding of rat antibody to G cell surface antigen (GCSA) of syngeneic AKR virus induced lymphoma cells was quantitated with the paired-radioiodine labeled antibody technique. With plots of titration data which conformed to linear equations derived from the mass action law, the following constants were determined: 1) The average surface density of antibody molecules bound to GCSA sites was 6.9×10^6 per cell and 11,300 per square micron. 2) The average equilibrium constant of the antigen-antibody association reaction was 6.54×10^6 liter $mole^{-1}$. 3) The mean binding affinity ($\Delta F°$) of the antigen-antibody bond was -9.17 Kcal $mole^{-1}$. 4) The rate constant for antibody dissociation at 0° was $3.67 \times 10^{-5} sec^{-1}$.

3. The plasma membranes of EB virus-infected lymphoid cells were isolated on sucrose density gradients in an attempt to isolate the EBV-coded membrane antigen (MA). Plasma membrane-associated HL-A and xenoantigens from these cells were readily isolated on the gradients by the procedure used. The location of antigens on the gradient was monitored by the presence of bound radioiodine-labeled proteins from antiserum and normal serum introduced by incubating the intact cells with

labeled proteins prior to fractionation (paired-label technique). Although the cells used were 40% positive for MA by membrane immunofluorescence prior to fractionation, no detectable MA remained associated with plasma membranes after fractionation. A peak of antibody-bound material in the 52-58% region of the sucrose gradient contained EBV by electron microscopy. We conclude that the MA appears to be loosely rather than covalently integrated into the plasma membrane of EBV-infected cells.

4. Spleen lymphocytes from tumor-immune animals completely inhibited the growth of tumor cells when mixed with tumor cells prior to inoculation. A ratio of 10 immune spleen lymphocytes per tumor cell was effective.

5. Splenectomy increases the resistance of mice to tumor transplant challenge.

6. Treatment of established tumors with immune spleen lymphocytes is in progress.

7. The methodology for pepsin digestion of antibodies to produce $(Fab)_2$ fragments, reducing the $(Fab)_2$ dimers, and recombining the monomeric units to make monovalent hybrid dimers was completed.

Significance to Biomedical Research and the Program of the Institute:

1. The finding that the immunogenicity of a tumor cells homogenate can be enhanced by prior infection with influenza virus has considerable potential for the immunotherapy of human tumors. Primary tumor tissue which is removed from the patient, infected with influenza virus, homogenized, inactivated with regard to infectivity, and then inoculated back into the patient, could conceivably enhance the patient's immune resistance to residual tumor and to the development of metastases.

2. The majority of animal and probably also human tumor cells possess antigens on their surface membranes which are capable of inducing a weak immunological rejection reaction in the host. The characterization of the density distribution of physiological behavior and metabolism of these tumor associated surface antigens (TASA) is important to the understanding of

the carcinogenic process and also to the development of immunological methods for the diagnosis and treatment of cancer.

3. The isolation of antigens in pure form from Epstein-Barr virus-infected lymphoid cells is a necessary prerequisite to the development of rapid and sensitive assays for the diagnosis and treatment of Burkitt lymphoma.

4. Work on the synthesis of hybrid antibodies will make available knowledge and techniques for studying the problem of blocking factors in serum which prevent tumor cell destruction by specifically sensitized lymphoid cells.

Proposed Course: Mechanisms of the influenza virus-mediated adjuvant effect in inducing tumor immunity will be intensively studied in the coming year. Methods of isolating the Gross cell surface antigen, already in progress, will be finished and reported next year. Attempts will be made to isolate antigens from lymphoid cells acutely infected with EB virus.

Honors and Awards: None

Publications:

Boone, C.W., Mantel, N., Caruso, T.D., Jr., Kazam, E. and Stevenson, R.E.: Quality control studies on fetal bovine serum used in tissue culture. In Vitro (In press).

Boone, C.W., Brandchaft, P.R., Irving, D.N., and Gilden, R.: Quantitative studies on the binding of syngeneic antibody to the surface antigens of AKR virus-induced rat lymphoma cells. International Journal of Cancer (In press).

Boone, C.W. and Blackman, K.: Augmented immunogenicity of tumor cell homogenates infected with influenza virus. Cancer Research (In press).

Serial No. NCI-4884
1. Viral Biology Branch
OASDVO, Etiology
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 - June 30, 1972

Project Title: Spontaneous and Virus-Induced Neoplastic Transformation In Vitro. Statistical Methods for Clinical Immunology

Previous Serial Number: Same

Principal Investigator: Dr. Mitchell H. Gail

Other Investigators: Dr. Charles W. Boone (NCI-4883)

Cooperating Units: None

Man Years:

Total:	2.25
Professional:	1.25
Other:	1.00

Project Description

Objectives:

1. To study factors influencing fibroblast motility in general.

2. To compare density inhibition of motility in a variety of tumorigenic and non-tumorigenic cell lines.

3. To explore the relationship between cell motility and cell division.

4. To develop statistical methods appropriate to the analysis of problems in clinical immunology.

Methods Employed:

1. Time lapse cinematography of cultured cells.

2. Statistical methods (computerized) specifically devised to measure cell motility and density inhibition motility.

Major Findings:

1. Colcemide decreases fibroblast motility, probably by causing the cell to lose its polarity and so decreasing the directional persistance with which it moves.

2. Cytochalasin reversibly inhibits fibroblast motility and does so at doses ten-fold less than those required to suppress cell division.

3. Procaine hydrochloride reversibly inhibits cell motility and cell division in pharmacologic doses, suggesting that cell motility depends, in part, on ionic permeabilities of the cell membrane.

4. Viral transformants of 3T3 fibroblasts, spontaneous transformants, and chemically induced fibrosarcoma explants all show markedly reduced density inhibition of motility compared to 3T3 fibroblasts. The degree of the diminution is not well correlated with tumorigenicity.

5. Statistical analysis of kidney transplant survival data allows the partition of risk of kidney loss into four clinically useful categories.

6. Statistical analysis of cardiac transplantation data casts doubt on the conclusion that this procedure prolongs life.

Significance to Biomedical Research and the Program of the Institute:

1. Invasiveness is a hallmark of the neoplastic process. Careful study of the basic determinants of cell motility will add to an understanding of the differences in invasive capacity between normal and tumorous cells.

2. Clinical decision-making according to the theories and rules of statistical analysis, supported by computerized data analysis, can aid the clinician in correctly evaluating clinical data to make better diagnostic and therapeutic decisions.

Proposed Course:

A thorough-going study of the relationships between density inhibition of motility and tumorigenicity in a large

number of spontaneous, virus-induced, and chemically induced cell lines, will be conducted.

Honors and Awards: None

Publications:

Gail, M.H. and Boone, C.W.: Effect of colcemide on fibroblast motility. Exp. Cell Res. 65: 221-227, 1971.

Gail, M.: Interpretative study of kidney transplant survival data. Transplantation 21: 194-201, 1971.

Gail, M. and Boone, C.W.: Cytochalasin effects on BALB/3T3 fibroblasts: Dose-dependent, reversible alteration of motility and cytoplasmic cleavage. Exp. Cell Res. 68: 226-228, 1971.

Gail, M.H. and Boone, C.W.: Cell-substrate adhesivity: A determinant of cell motility. Exp. Cell Res. 70: 33-40, 1972.

Gail, M.H.: Does cardiac transplantation prolong life? A reassessment. Ann. Int. Med. (In press).

Gail, M.H. and Boone, C.W.: Procaine inhibition of fibroblast motility and proliferation. Exp. Cell Res. Accepted for publication.

Serial No. NCI-4888
1. Viral Biology Branch
OASDVO, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Molecular Aspects of Viral Oncology

Previous Serial Number: Same

Principal Investigator: Dr. Timothy E. O'Connor

Other Investigators: Dr. Emerson Chan, VBB, NCI, Visiting Scientist (NCI-4894)

Cooperating Units: None

Man Years:

Total:	3.3
Professional:	1.0
Other:	2.3

Project Discription

Objectives:

To explore the microbiology of a feline sarcoma-leukemia virus complex in cell culture systems derived from various animal species with a view to providing information and experimental systems useful in the detection and isolation of new sarcoma and/or leukemia viruses of other species including man.

Methods Employed:

Cell culture systems derived from various mammalian species and cell-transformation assays in these systems.

Major Findings:

The Snyder-Theilen feline sarcoma virus complex was shown to consist of a cell-transforming virus and an excess of a non-transforming (leukemia) virus. The non-transforming virus could act in some cell culture systems

as a helper virus for the defective transforming virus but cell cultures could be rendered resistant to infection with the transforming virus by prior inoculation with sufficient non-transforming virus. The S-T virus complex could infect cat, bovine or human cells. Serial passage of the virus complex in bovine cells ultimately selected a virus population showing significantly enhanced plating efficiency on bovine as compared to feline cells. Various individual foci of virus-transformed bovine cells could be grown to mass cultures which shed substantial, moderate or essentially no progeny virus. The properties of such minimal virus shedding transformed cultures and their usefulness as detector systems for other leukemia viruses are being further explored.

Significance to Biomedical Research and the Program of the Institute: Knowledge of the behavior of known animal sarcoma and leukemia viruses in cell culture systems is essential as a guide to the detection and propagation of suspected human oncogenic viruses in tissue culture.

Proposed Course: This study will be continued and extended in human cell culture systems. It is expected that Dr. O'Connor's participation in experimental phases of this study will shortly cease due to assignment to other duties.

Other Activities: As Head, Molecular Virology, in the Office of the Chief, VBB, Dr. O'Connor advised the Chief, VBB, on various molecular aspects of research activities within the Branch and of developments in molecular aspects of viral oncology.

As Advisor in Molecular Biology to the Special Virus Cancer Program, Dr. O'Connor advised the Associate Scientific Director for Viral Oncology on the molecular aspects of viral oncology as they pertained to the SVCP.

In September, 1971, Dr. O'Connor was designated jointly by the Scientific Director of Etiology and Scientific Director of Chemotherapy to coordinate scientific aspects of a collaboration between NCI and Dow Chemical Company aimed at screening the usefulness of various Rifamycin SV derivatives as antagonist of cellular enzymes, oncogenic viruses and tumors. Together with Dr. Harry Wood, Jr. (Chemotherapy), he has supervised a program of drug testing and synthesis under this cooperation.

Honors and Awards:

Dr. O'Connor served as a member of the Program Committee of the American Association for Cancer Research, 1972.

Lectures were presented by invitation on various aspects of cancer research to the Candlelighters Society, Washington, D. C., December, 1971, The University of Pennsylvania, Philadelphia, Pa., January, 1972, the University of Virginia (George Mason College), Fairfax, Va., February, 1972, and the University of Maryland (Dental School), Baltimore, Md., March, 1972. He has been invited to address the American College of Physicians in June, 1972.

Publications:

Rauscher, F. J., Jr. and O'Connor, T. E.: Cancer Viruses. Holland, J. F. and Fry, E. (Ed.): Cancer Medicine. Philadelphia, Pa., Lee and Febiger, 1972. In press.

O'Connor, T. E.: Reverse transcriptase. Dutcher, R. (Ed.): 5th International Symposium on Leukemia and Lymphoma Research. Basel, Switzerland, Karger, 1972. In press.

Serial No. NCI-4889
1. Viral Biology Branch
OASDVO, Etiology
2. Microbiology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Investigation of Factors which Modify Oncogenic Virus Infection and Oncogenicity both in vivo and in vitro.

Previous Serial Number: Same

Principal Investigator: Dr. Willie Turner

Other Investigators: None

Cooperating Units: Inside NIH

Dr. Dharam Ablashi, VBB, NCI (NCI-4834)

Man Years:

Total:	1.5
Professional:	0.5
Other:	1.0

Project Description

Objectives:

(A) To characterize Rauscher murine leukemia virus produced in human embryo kidney cells.

(B) To study the interaction of DNA and RNA tumor viruses in vitro.

(C) To investigate the effect of a C-type virus of the rat on replication of mouse leukemia virus.

Methods Employed:

Established virological and immunological methods were utilized to titer and identify the viruses under study.

Major Findings: A semi-micro XC cell assay for murine leukemia virus (MuLV) was developed using microcell cultures in plastic plates. Although titers obtained are about one log lower than those observed by the conventional procedure, the method provides a rapid, reproducible in vitro method for comparative assay of multiple samples of MuLV and neutralizing antibodies.

Rauscher leukemia virus (RLV) recovered from the plasma of infected mice had been shown to be infectious for human cells by other investigators. Studies to compare RLV grown in human and in mouse tissue showed that continuous propagation in human cell cultures did not affect the synthesis of mouse virus group specific antigen, but did eliminate infectivity for mouse cells and ability to induce the typical disease in animals. Cross-neutralization tests indicated that the viral progeny from both cell systems contain a common coat antigen suggesting a close realationship.

The interaction between SV40, a DNA virus, and RNA mouse tumor viruses was examined in vitro. A continuous line of mouse macrophages, transformed by SV40, was obtained. Although these cells did not shed virus, they contained the SV40 t-antigen but not the v-antigen. Superinfection of these cells with Moloney leukemia virus (MuLV-M) yielded a chronically infected cell system which continued to produce virus at a titer of 10^3 to 10^5 ID_{50} per ml. In contrast, these cells, infected with Moloney leukemia virus and mouse sarcoma virus (MSV-M), gave progressively lower titers of MSV-M, but retained elevated titers of MuLV-M, through succeeding sub-cultures. This phenomenon did not appear to be due to interference by the helper leukemia virus. Mice, inoculated with MSV-M from this cell system, showed not only solid tumor induction, but also an atypical syndrome which consisted of the development of hemorrhagic lesion in the thorax of the mice. The neutralization and in vivo data obtained suggested that the RNA virus from the persistently infected SV40 culture was identical to the progenitor virus. However, the difference in pathology seen in mice infected with the MSV-M from this culture suggested that some alteration in the virus had occurred. Further, preliminary data indicated that SV40 v-antigen was produced in the culture after infection with MSV-M and infectious SV40 was detected in the culture fluids.

Tumor cell cultures from rats with mammary carcinoma, such as the R-35 and W/Fu T lines, shed C-type virus particles. When such cells and appropriate control cultures of rat tissue free of C-type particles are infected with equal multiplicities of MuLV, the yield of MuLV is more than 4 logs higher in the tumor lines than in control lines. This suggests potentiation of MuLV by a rat C-type particle. The progeny virus has been shown to be MuLV. The significance of this observation is not yet understood and further research is warrented.

Significance to Biomedical Research and the Program of the Institute:

Comparatively little attention has been directed to explore the effect of dual infections by tumor viruses on the cell and animal host. Our studies show that important modifications in host response and in virus expression may ensue as a result of intracellular interactions between co-infecting viruses.

Proposed Course:

This project was terminated with the departure of the principal investigator.

Honors and Awards:

None.

Publications:

Turner, W., Ebert, P., Riechers, L., Pearson, J.W., and Chirigos, M.A.: Elucidation of the nature of the murine oncogenic virus inhibitor isolated from JLS-V5 cell line. Proc. Soc. Exp. Biol. and Med. 138, 3: 1030-1034, December, 1971.

Bass, L.A. and Turner, W.: A semi-micro XC cell assay technique for murine leukemia virus. Applied Micro. 23: 200-201, February, 1972.

Rice, J.M., Turner, W., Chirigos, M.A., and Spahn, G.: Dose responsiveness and variation among inbred strains of mice in production of interferon after treatment with Poly (I) · Poly (C) [Poly-D-Lysine] complexes. Applied Micro. 22: 380-386, September, 1971.

Serial No. NCI-4890
1. Viral Biology Branch
OASDVO, Etiology
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 - June 30, 1972

Project Title: Immunochemical and Biochemical Analysis of Cell Lines Derived from Methylcholanthrene-Induced Murine Fibrosarcomas

Previous Serial Number: None

Principal Investigator: Dr. Thomas W. Orme

Other Investigators: Dr. Charles W. Boone (NCI-4883)

Cooperating Units: None

Man Years:

Total:	1.75
Professional:	0.75
Other:	1.0

Project Description

Objectives:

1. To chemically define the nature of TSTA in MCA-induced murine fibrosarcoma cell lines.

2. To investigate the mechanism of augmentation of TSTA immunogenicity which follows influenza virus infection of MCA cell lines.

3. To prepare cell-free TSTA.

4. To describe the potential usefulness in cancer diagnosis and therapy of purified, characterized tumor-specific antigens.

Methods Employed:

1. Tumor-graft rejection assays and radioisotopic foot-pad assays for delayed hypersensitivity to tumor cells.

Serial No. NCI-4891
1. Viral Biology Branch
OASDVO, Etiology
2. Electron Microscopy Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Recovery and Biological Characterization of Viruses Associated with Neoplastic Diseases.

Previous Serial Number: None

Principal Investigator: Dr. Herbert K. Oie

Other Investigators: Dr. Harish C. Chopra (NCI-4874)

Cooperating Units: Inside NIH
Dr. D. V. Ablashi, VLLB, NCI (NCI-4834)
Dr. A. Gazdar, VLLB, NCI (NCI-4824)
Dr. G. Pearson, VBB, NCI (NCI-4880)

Outside NIH
Dr. D. Fine, Bionetics Research Laboratories, Kensington, Maryland
Dr. P. A. Fabrizia, Bionetics Research Laboratories, Kensington, Maryland

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description

Objectives:

1. To isolate and characterize viruses from tumor tissues and tumor-derived cell cultures.

2. To study biological properties of the R-35 virus recovered from mammary carcinoma of the rat particularly its possible role in the genesis of rat mammary cancer and its potential to transform.

Methods Employed:

Established techniques for cell culture, cytogenetics, and electron microscopy were used. Cultures of selected tumors were treated with IUDR and DMSO, or subjected to other procedures, to attempt to activate production of virions detectable by electron microscopy.

Major Findings:

1. Two mammary carcinomas and an osteosarcoma of human origin were cultured and treated with a combination of IUDR and DMSO. The treated cells showed no evidence of virus when examined by electron microscopy. No virus was detected in a spontaneously transformed monkey embryo cell culture after similar treatment. Co-cultivation of normal dog embryo cells with mammary tumor of the dog also failed to show evidence of an associated virus.

2. The R-35 virus recovered from a rat mammary tumor (Chopra et al, 1970) resembles the C-type viruses in its morphology. This virus was reported to transform cultured normal rat mammary tissue (Ahmed et al, 1972). This was verified by the observation of focal areas of transformation after infection of rat mammary cells. No evidence of viral reproduction was obtained following exposure to infection of human NC-37 lymphoblasts or cell cultures from hamster embryo, hamster mammary gland, monkey embryo, or dog embryo. The transformation of rat mammary gland cell cultures was inefficient and inconsistent. Selection of cells from different focal areas of transformation provided two lines of cells which grew as tumor when implanted into rats. Histopathologically, these tumors resembled carcinomas. Virus was produced by these tumors as well as by the transformed cells in culture. Immunofluorescence tests and electron microscopic examination showed 10-20 percent of the cells producing virus at a given time.

3. A cell line derived from a rhesus monkey embryo culture which had spontaneously transformed is being investigated. The cells in the monolayer appear epithelioid with some giant cells present. These cells grow more rapidly than do normal cells and pile up when confluency is reached. Cytogenetic analysis showed 25 percent polyploidy, no modal number, and a unique second marker chromosome. To determine whether activation of a virus was responsible for the transformation, the cells were treated with IUDR and DMSO. Electron microscopic observations prior to and after this treatment failed to detect particulate virus.

Significance to Biomedical Research and the Program of the Institute:

These studies were initiated to apply newer techniques known to induce virus replication in covert infections by RNA tumor viruses to the mammary tumor problem. In animal systems, the mouse mammary tumor virus has been a lone representative of RNA viruses which induce neoplasia in epithelial

cells. The possibility that a new virus, differing morphologically from the mouse mammary tumor virus, may induce rat mammary tumors is most important as a system for evaluating interrelationships between these agents. Its activity in eliciting transformation of epithelial cells, if verified and established in a reproducible system would contribute substantially to other studies involving human mammary carcinogenesis.

Proposed Course:

The studies described above will be continued. In addition, intensive efforts will be directed to determine possible viral relationships to human and dog mammary tumors.

Honors and Awards: None

Publications:

Chopra, H. C. and Oie, H. K.: Possible etiological role of virus particles in rat and monkey mammary carcinomas. J. Natl. Cancer Inst. (In press)

Serial No. NCI-4892
1. Viral Biology Branch, OASDVO, Etiology
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 - June 30, 1972

Project Title: Mechanisms of the Cellular Immune Response to Tumors

Previous Serial Number: None

Principal Investigator: Dr. Meera Paranjpe

Other Investigators: Dr. Charles W. Boone (NCI-4883)

Cooperating Units: None

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description

Objectives:

To determine mechanisms of the cell mediated immune response to tumors.

Methods Employed:

1. Radioisotopic footpad assay for delayed hypersensitivity in the mouse (developed in this laboratory).

2. Radioisotopic methods for labeling cells and molecules.

3. Immune cell reconstitution of thymectomized x-irradiated mice.

Major Findings:

1. The inoculation of SV40-transformed and methylcholanthrene-induced tumor cells into the foot pad of tumor-immune mice induces a delayed hypersensitivity reaction easily measured by the escape of I-125

labeled serum protein into the interstitial fluids of the inflamed area. Passive transfer of this reaction was accomplished with spleen cells but not with serum from tumor-immune animals.

2. Spleen cells from tumor bearing animals were not active in adoptive transfer of the delayed hypersensitivity reactions to tumor cells, indicating that an immunologically tolerant state develops in the tumor bearing animal.

Significance to Biomedical Research and the Program of the Institute:

Analyzing the cell-mediated immune response to tumors provides leads to ways of manipulating the immune response to the advantage of the cancer patient. It also gives information on the complex, physiological process of inflammation in general at the cellular level.

Proposed Course:

The development of tolerance in the tumor bearing animal and methods for abrogating it, will be studied. Cooperation between different cell types required for the delayed hypersensitivity reaction to tumor cells will be analyzed. The adjuvant effect of inducing cotemporal delayed hypersensitivity reactions, particularly those to mycobacteria, on the tumor immune response, will also be pursued.

Honors and Awards: None

Publications:

Paranjpe, M.S. and Boone, C.W.: Delayed hypersensitivity to SV40 tumor cells in BALB/c mice demonstrated by a radioisotopic foot pad assay. J. Nat. Can. Inst. 48: 563-566, 1971.

Serial No. NCI-4894
1. Viral Biology Branch
OASDVO, Etiology
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on DNA (Herpesvirus saimiri) and RNA (feline sarcoma) tumor viruses.

Previous Serial Number: None

Principal Investigator: Dr. Emerson W. Chan

Other Investigators: Dr. Timothy E. O'Connor (NCI-4888)
Dr. Virginia C. Dunkel (NCI-4881)

Cooperating Units: None

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description

Objectives:

1. To examine the host range and composition of the Snyder-Theilen strain of feline sarcoma virus.

2. To develop micro-assay techniques for Herpesvirus saimiri and to determine physico-chemical characteristics of this virus.

Methods Employed:

1. Mass cell culture and focus-selection techniques were used in studies on feline sarcoma virus. Standard virological procedure were applied to study infection and transformation of mammalian cells by this virus.

2. Assay of Herpesvirus saimiri under methyl cellulose in microtest plastic plates was developed. Radioisotope labeled thymidine was used to prepare labeled virus for further physical characterization.

Major Findings:

1. Feline Sarcoma Virus - The host cell range and composition of the Snyder-Theilen strain of FSV were examined. Infection of feline embryonic lung, human embryonic lung, or bovine embryonic trachea (BT) cell cultures with FSV induced morphological transformation and the production (with terminal dilutions of FSV) of discrete focal lesions. In each case, cultures inoculated with higher dilutions of FSV gave on further passage non-transformed cultures which resisted superinfection with FSV and which shed a virus capable of inducing interference. Focus production by FSV in BT cultures was "two-hit" but became apparently "one-hit" on coinfection with the accompanying virus propagated in BT cultures. Mass cultures of transformed BT cells grown from individual foci were found to shed various levels or no focus-inducing virus. BT cultures transformed with high inocula of FSV initially shed virus having an unaltered host range, but after many months passage the culture shed virus infective for BT or human cells; but not for cat cells. These data indicate that FSV stocks contain a defective focus-inducing virus and an excess of a non-transforming virus.

Preliminary virus-rescue experiments indicated that one of the transformed but non-virus-producing BT cell cultures could be induced to produce transforming virus when the culture was superinfected with feline leukemia virus.

2. Herpesvirus saimiri - An efficient and reproducible microtiter plaque assay was developed for HVS. Studies on the cell-virus relationship indicated that the virus was not strictly cell-associated. A single sharp peak of infectious virus at a density of 1.18 was obtained after centrifugation of a virus preparation, prelabeled with H^3-thymidine, in a sucrose density gradient. The peak of infectious virus co-incided exactly with a major peak of radioactivity.

Significance to Biomedical Research and the Program of the Institute:

The infection and transformation of various mammalian cells by feline sarcoma virus was conducted to obtain transformed, non-virion-producing mammalian cells which could serve as detectors for leukemia viruses in animals and man. If successful, such a system would provide a valuable tool for leukemia research.

Herpesvirus saimiri induces lymphosarcoma and lymphocytic leukemia in different non-human primate species. In this respect it is an important system for the determination of the significance of herpesviruses in oncogenic processes. The studies completed provide a convenient method for reproducibly titering a large number of virus samples. This method was developed to permit the initiation of studies to physically and chemically characterize this herpesvirus.

Proposed Course:

These studies will be terminated.

Honors and Awards:

Postdoctoral fellowship of the National Cancer Institute of Canada, July 1, 1970 through March 31, 1972.

Publications: None

SUMMARY REPORT

VIRAL CARCINOGENESIS BRANCH

July 1, 1971 June 30, 1972

Introduction

The cancerous state begins when a stable neoplastic (genetic) change occurs in one or more normal cells. This first step is attributable, according to modern theory of the genetic code, to a defect or breakdown in normal cell regulation of inherited oncogenic genes (oncogenes), the structural gene that initiates and maintains the "spontaneous" neoplastic state. The tumor actually develops from the transformed neoplastic cell as a consequence of a second step occasioned by defects or breakdowns of the host's immunological surveillance, thus allowing neoplastic cells to replicate more or less ad lib in the whole organism.

Research Objectives of the Viral Carcinogenesis Branch.

The inhouse Viral Carcinogenesis Branch and the Solid Tumor Segment's contracts program, working in close collaboration with other SVCP Branches and Segments, are engaged in comprehensive studies of the etiological factors involved in the two events described above which, along with exogenous carcinogenic factors, are critically important in the development of cancer.

Prevention or repair of the breakdowns in regulation and control at both the cell and organism level is of course our ultimate goal. This requires studies of the normal cell and of the cancer cell, and of exogenous agents that impinge on or alter normal cell behavior, and the host responses to this alteration.

Our contemporary strategy is to prevent cancer first in experimental and natural animal systems and then, shortly afterwards, in man. In order to accomplish this we have developed several staging areas for collaborative research.

Specific Contemporary Targets of VCB-STS Programs:

1. To test the validity and heuristic value of the RNA viral oncogene hypothesis in humans as well as in laboratory and feral vertebrates. Very recent data (late FY 1972) provided powerful direct support for the viral oncogene hypothesis at the cellular level (*vide infra*).

2. To apply the findings and concepts generated in Target 1 to the development of better techniques capable of providing more accurate assessments of the specific structural and regulatory genes and other endogenous mechanisms involved in spontaneous neoplasia.

3. To apply new *in vitro* as well as *in vivo* carcinogenesis techniques developed in VCB and STS in quantitative assays of unknown as well as known environmental carcinogens. In the process we are studying carcinogenic (and mutagenic) agents as (a) inducers of expressions of the endogenous RNA tumor virus genes, and (b) co-carcinogenic agents acting in concert with additional overt copies of infectious (but nontransforming) RNA tumor viruses, the latter serving as critical determinants of *in vitro* neoplastic transformation.

4. To utilize the remarkable increase in sensitivity provided by cells infected with nontransforming RNA tumor viruses to screen for and quantitatively assay for carcinogens suspected to be extensively present in the random ecologies in which highly developed societies live.

5. To apply newer knowledge and techniques in attempts to prevent oncogenic transformation and tumors and conceivably also to find ways to reverse the oncogenic state at the cellular level.

6. To make use of recent findings by STS-SVCP scientists which show that the immunological system exercises critical controls over tumor cell growth in vivo. Cell mediated and humoral responses to tumor cells have been demonstrated.

7. To continue the search for overt representatives of the postulated RNA tumor virus of man; at least one very good candidate has been isolated by McAllister and his associates. This virus grows well in culture and has been characterized as a mammalian type C RNA virus. Thus concepts and techniques developed from our extensive natural history studies of similar viruses in animal systems are proving very useful in similar studies of human cancer.

8. To develop comprehensive epidemiological and laboratory studies of human cancer in a large urban population (Los AngelesCounty), including surveillance not only of human cancer incidences and cancer virus expressions, but of the similar type C viruses found in several species of feral animals present in large numbers in the same ecosystem. Identification of carcinogenic factors within the Los Angeles ecosystem and their influence on cancer incidence are major goals.

New Discoveries Relating Specifically to the Eight Specific Research Targets of VCB and STS

I. In support of the viral oncogene theory.

The reports late in 1971 by Rowe and Klement and their associates that all clones and subclones of mouse and rat cells can be induced by mutagenic and carcinogenic chemicals (BrdU, IdU, 3MC, DMBA and others) to replicate infectious type C RNA viruses can only be explained by universal genetic transmission of the complete RNA type C genome. Similar observations by Aaronson and Todaro (VLLB) in all clones of nonpermissive BALB/c and Swiss 3T3 mouse cells provided simultaneous confirmation of these exceedingly important findings. Entirely compatible data were also supplied for hamster cells by Kelloff and Freeman, and for chicken cells by Weiss, Hanafusa and Vogt. We interpret these new data, as well as recent reports of type C viral molecular RNA sequences in virus-free normal and tumorous tissues of mice and chickens (STS contractors Green, Duesberg, Bishop and Levinson) as direct evidence for inheritance of the type C RNA genome by all the cells of these vertebrates.

II. A. Endogenous mechanisms. Mendelian studies of endogenous virus expressions.

Mendelian genetic studies by Rowe and Hartley, Lilly and Meier and their associates of RNA tumor virus genomes in inbred mouse strains clearly established that natural (endogenous) gene expressions of RNA tumor viruses are controlled by a number of identifiable dominant and recessive genes. Meier's group has identified two allelic genes, one in which the dominant allele specifies group-specific antigen expression (gs+); the other, the allelic recessive gene specifying gs- or the switched off state. Similar V+ and V- alleles specify the production or non-production of viruses. Host genes controlling gs antigen proteins of avian RNA viruses were reported earlier by the British workers Payne, Chubb and Weiss.

Taylor and Meier have now derived recombinant inbred lines of mice from the mendelian crossing experiments using AKR (gs+,gs+) and C57L (gs-,gs-) having the following gs and V characteristics: (1) gs+,V+; (2) gs-,V-; (3) gs+,V-; (4) gs-,V+. Only line #1 has infectious virus. By crossing virus-negative lines 3 and 4, infectious virus is produced and released. This is best described as genetic rescue of the virus.

More recently Meier and Taylor have found that another strain of inbred mouse has a dominant gene specifying gs1: the F_1 cross AKR results in no gs expressions, and of course no virus expression. Thus by concentrating, in any strain or breed, genes for switching off gs antigen as has been done in the inbred mouse system, cancer could be virtually eliminated from such animals. While not directly applicable to the solution of human cancer, it provides enormously important concepts concerning the decisive nature of identifiable natural regulating genes in determining the incidence of cancer. This view was further supported by the results of F_1 backcross in the AK x L matings; the offspring at 20 months developed 102 tumors as follows: 91 tumors occurred in mice whose spleens were gs+ at birth. Nine of the 11 tumors from gs- mice (at birth) were gs+.

These data definitely suggest a linkage between the gs+ gene and the oncogene. The noninfectious gs expressions transmitted and controlled through succeeding generations can only be explained on the basis of genetic inheritance; thus infectious virus genomes have no need to be transmitted horizontally. These mendelian studies clearly establish the validity of the RNA viral oncogene theory so far as the inbred mouse is concerned.

II. B. Endogenous mechanisms. Molecular hybridization.

Molecular hybridization experiments employing the DNA products of avian and murine viruses and DNA of normal and tumor cells show that DNA copies of the virus produced by the reverse transcriptase sequences are present in normal as well as tumor cells, regardless of whether or not they have infectious virus. [Dr. Green, in HT-1(MSV) hamster cells; Duesberg, Bishop, Varmus and Levinson in avian cell systems; Scolnick, Aaronson and Parks in

virus-free mouse sarcoma cells]. We conclude from these findings that normal cells contain part or all of the sequences necessary to make RNA viruses.

III. A. Mechanisms of exogenous inducers of oncogenesis: RNA tumor viruses as determinants of transformation.

In vivo studies: In 1971 our associates (Whitmire, Salerno, Meier, Myers, Peters et al) and we have reported simultaneous activation of type C RNA tumor virus expressions (most often gs antigen, occasionally infectious virus) in chemically induced as well as spontaneous tumors in extensive surveys of many different inbred and outbred strains of mice. With the development in FY 1972 of the extremely sensitive radioimmune precipitation inhibition (RIPI) test developed by Parks and Scolnick, and Gilden and Oroszlan, many or most of the tumors which were gs negative by complement-fixation (CF) are now shown to be positive for gs antigen. We concluded from this that the coincident switch on of the major species-specific protein peptides of the RNA virus in tumor cells produced by a variety of chemical carcinogens (3MC, DMBA, BP and nitrosamines) provided evidence that the carcinogens act to derepress the RNA viral genome known to be present in mouse cells.

III. B. Evidence that activated "non-transforming" viruses carry oncogenic information. (Peters et al; Freeman, Price and Zimmerman et al)

Viruses activated and recovered from normal and tumorous mouse tissue, and from mouse cells (normal and transformed) grown in tissue culture were tested for oncogenicity in various ways. When injected into newborn mice of the isologous strain, up to 70% of the inocula have produced leukemia by 15 months; these experiments are still underway. Many such viruses are also being inoculated into genetically homologous and heterologous cell systems, following which they will be observed for many additional subcultures for evidence of spontaneous transformation (in comparison, of course, with controls); although extensive tests are still in progress, previous observations in various RNA virus-infected and uninfected mouse, rat and hamster cells revealed in most instances that spontaneous transformations occurred much earlier in infected cells; the onset of transformation often being determined by the genotype of the cells. We conclude from these observations and from numerous similar observations by others that wild type RNA viruses and their subinfectious virus expressions have oncogenic potential.

III. C. Activation of viral oncogenes in vitro.

Perhaps the most rapid and definitive test for oncogenic potential is one in which a given dose of RNA virus is added to mouse, rat and hamster cells together with small doses of carcinogenic chemicals (3MC, DMBA). As reported by Rhim, Freeman and Price, cells are readily transformed (from 9 days to 5 weeks) after adding both virus and chemicals; when only one is added, no transformation is observed, the cells behaving like untreated, uninfected cells. Recently it has been found that the dosage of input virus as well as titers of established virus present in the cells at the time of

treatment with 3MC were decisive determinants of transformation (Price and Zimmerman). If the virus level was less than 1.5 logs/ml of 10% cell pack extract, the cells generally were not transformed; higher virus titer levels apparently leads to increasing efficiency of transformation. Also, if the 3MC is added to the cells 24 hours before virus, transformation does not ensue. Recent experiments show that the presence of infectious virus does not increase permeability of the cells to the chemicals (Zimmerman).

We conclude from this that the added infectious RNA viruses provide large numbers of oncogenes available for derepression by the action of the carcinogenic chemicals.

IV. Rapid in vitro tests for environmental carcinogens based on prior infection of rodent cells with RNA tumor viruses.

Following up FY 1971 studies which indicated that rat and hamster cells infected with RNA tumor viruses provided extremely rapid sensitive and reproducible transformation assay systems for carcinogenic chemicals, Freeman and Price in FY 1972, working with Weisburger and others, tested fairly large numbers of non-carcinogenic analogs together with their related carcinogens in a standardized rat Rauscher leukemia virus-infected cell system. The positive and negative results obtained agreed remarkably well with the known in vivo carcinogenic activity of the chemicals tested. These and additional specimens are now on test in Dr. Rhim's virus-infected mouse and rat cell systems. In comparative tests, Rhim's Swiss mouse cell + AKR virus system was found to be the most sensitive of the various test systems, often yielding transformation endpoints within 2 to 3 weeks.

Effects of smog and tobacco smoke fractions: Freeman and Rhim separately tested (in their respective rat and mouse virus infected cell systems) several smog residues extracted in benzene and methanol and a number of fractions of tobacco smoke. All of the smog residues were profoundly oncogenic, showing 100 to 1,000 times more activity than the benzo(a)pyrene (BP) equivalents demonstrated in the smog preparations. Similarly, within relatively short intervals, Rhim found that 4 fractions of tobacco smoke were active as transforming agents in his mouse-AKR virus system. These fractions were among the 5 or 6 fractions furnishing significant numbers of epidermal tumors in mice as tested by Dr. Bock. The two strongest fractions in vivo were also the strongest transforming agents in Rhim's in vitro tests.

We conclude from these studies and previous studies not described here that Freeman's and Rhim's virus-infected in vitro test systems will have a definite and possibly a major role to play in identification and quantitative assays of chemical carcinogens in the environment.

V. Efforts to prevent endogenous RNA virus genome specified tumors.

In FY 1972 we embarked on serious attempts to search for agents that would have the best prospects for preventing or modifying cancer presumed from our other studies to be due primarily to endogenous but identifiable RNA virus gene products. We are studying natural cell repressors (Portugal and Simonds), interferon (Whitmire, Salerno), rifamycin derivatives (Green, Salerno, Rhim) as well as viral and cellular vaccines (Girardi and Whitmire).

All results are preliminary. However, Portugal and his associates have produced and fractionated cellular components from normal mouse cells carrying large amounts of Rauscher leukemia virus, which have shown moderate antiviral and antisarcoma effects on XC plaques and in vivo MSV tumor inductions. This promising lead will be followed up by tests against transformation in virus-free cells.

Salerno, Spahn and Whitmire have shown that high titered murine interferon given 3X a week completely prevented subcutaneous sarcomas normally inducible (at 80-90% level) by 3MC; the mouse used for this experiment was the outbred CF-1 strain which contains moderate amounts of overt RNA virus infection. The experiment is now being repeated using strains of mice that do not have viral expressions prior to tumor induction. We conclude from these results that the endogenous RNA virus genome was involved in the tumors induced by 3MC, since the action of interferon is strictly antiviral. Obviously interferons must be explored more intensively as anti-tumor agents.

A number of rifamycin derivatives having more activity against the RNA virus reverse transcriptase than against cell DNA polymerases have been identified by Drs. Green, Hackett and Calvin. Although moderately active against murine sarcoma viruses in vitro, the critical tests of these and future anti-polymerase products (as with interferon) will be in carcinogen-induced transformations and tumors in cells and animals in which overt virus is not normally expressed. Our associates have such systems and they will look for efficacy in them in cooperation with Green and Hackett.

We conclude that the evidence for efficacy of rifamycins or rifampicins in oncogenic test systems free of infectious virus is as yet non-existent; obviously until studies in such systems are proven positive, any claims for efficacy are exceedingly dubious.

Girardi has confirmed the finding by Coggins and his associates that "vaccine" preparations of embryonic hamster tissues have suppressive effects on hamster tumors; however both found effects only in male hamsters, suggesting effects other than specific immunogens. In a preliminary experiment, Whitmire and associates found that crude cell extract vaccines had a moderate suppressive effect on tumors induced by 3MC; additional experiments are in progress. Our present view is that anti-RNA tumor virus vaccines are unlikely to have much effect on natural or chemically induced cancers in natural situations (switched off for RNA virus expressions), at least where cancer develops

in the absence of overt RNA virus infections. However this assumption should not prevent relatively easily performed additional experiments.

VI. Immunological studies.

The influences of immunological surveillance mechanisms on cancer incidence in animal systems are well documented. This is particularly evident in the inbred mouse study systems where the effects of thymus removal and treatment with immunosuppressants (Imuran and anti-thymic or anti-lymphoid cell sera) leads to increased spontaneous cancers as well as those induced by viruses or chemicals (Meier and others). In chickens and in mice we and our associates have observed that high natural antibodies to the type C RNA virus greatly reduces natural virus expressions and cancer incidences.

Recent studies of the Hellstroms of cell mediated and humoral immunity against human cancers have been extremely productive. First of all they have shown that 80% of all cancer patients have detectable immunity against their own cancers and many to the same type of "organ-specific" tumors in other cancer patients. More recently they discovered that patients with growing cancers developed antibodies that block protective tumor cell cytotoxicity. When tumors were removed or otherwise responsive to therapy, they discovered another category of antibodies,"unblocking" antibodies that abrogated the blocking antibodies. Sjogren and his associates, working with the Hellstroms, demonstrated both blocking and de-blocking antibodies in sera of rats carrying tumors induced by polyoma virus. Studies of the effect of blocking and particularly de-blocking antibodies on tumors induced in vivo by carcinogenic chemicals represent an important next step. The importance of such observations for possible immunotherapy and for predicting outcomes of other types of therapy are obvious.

VII. The search for specific human RNA tumor viruses (types C and B).

After years of searching for type C RNA tumor viruses in leukemias and other tumors of man and some otherwise unconfirmed "sightings" of unidentified "particles", as of July 1971 no viable candidate human type C viruses were available for study.

During FY 1972, the isolation of two bona fide candidates for human type C viruses were reported by two different SVCP-supported contract groups.

The ESP virus: The type C virus in the ESP human cell line reported by Priori and her associates was found by various investigators in SVCP including 3 in the VCB-STS programs to have mouse specific gs antigens. Two other investigators found the antigenicity of RNA dependent DNA polymerase (RDP) to have the properties of the mouse RDP. Since the properties of ESP virus have not been shown to be much different from the host cell modified viruses (RLV, KiMSV) grown in many different human cells, the true origin of this virus remains in doubt.

The type C RD114 virus: This virus, which remains the best human specific candidate, originated in McAllister's RD human rhabdomyosarcoma cells after passage of this cell line by Drs. Gardner and Officer through a fetal cat. The virus observed in the RD114 cells was at first assumed to be FeLV of the cat; however, subsequent intensive studies by 6 different VCB and STS groups revealed that the virus had none of the species specific gs-1 properties of FeLV or for those of the mouse, rat, hamster, chicken, or viper type C viruses. Also, while the envelope antigens and polymerase antigens were entirely unique and distinct from that of other available viruses, the gs-3 antigen of RD114 was the same as that of the mammalian interspecies specific antigens. The RNA $\longrightarrow$ DNA polymerase of RD114 was shown by Parks and Scolnick to be related to those of the woolly monkey and gibbon ape, thus suggesting that there may be common antigens in addition to gs-3 shared by a "primate group" of viruses. Although the RD114 is the best available candidate for a human type C RNA virus, conclusive evidence is not yet available. It will be necessary to demonstrate gs and other species specific antigens in additional type C isolates from human tissues or alternatively in human tumor cells. Because overt expressions of the human type C virus can be expected from many experiences to be rare, studies of viral RNA $\longrightarrow$ DNA hybridization with known tumor cell RNA or DNA may be required.

Hybridization studies with cat virus DNA by Baluda and Roy-Burman show no significant homologies with RD114 virus. Studies by M. Green (St. Louis University) in hybridizations between RD114 DNA and RNA's of Hodgkin's cancer cells have suggested an exceptionally high degree of homology. Since these studies are preliminary they will require much confirmation before the human nature of the RD114 virus can be accepted.

Type B RNA tumor viruses and natural history studies in humans and in mice.

VCB and STS scientists Parks, Gilden, Gardner, Henderson, Roy-Burman, Rongey and Zeve are collaborating with Spiegelman and Schlom in studies of mammary tumor viruses (MTV) in both human and mouse milk specimens. All available new and old techniques are utilized including tests of milk for RDP, type B particles and group-specific antigens. The collaborative effort is focused on the human mammary tumor problem which is dependent on the USC cancer surveillance study program (Henderson and associates) for locating and supplying milk specimens from lactating women who are daughters of cancer patients. Task 2 is electron microscopic examination by Gardner, Rongey and Zeve for type B (or C) particles. Task 3 is processing of the fresh milk specimens for RDP tests by Roy-Burman and his associates. Task 4 involves the actual testing for RDP by Spiegelman and Schlom, Parks and Scolnick, and Roy-Burman.

VIII. Comprehensive field and laboratory studies of the etiology and epidemiology of human and animal cancers.

In FY 1971 the USC contract study program, now in a new building furnished by USC (20,000 sq. ft.), was expanded to include surveillance of all human cancers in Los Angeles County. This cooperative program now includes extensive participation by USC Departments of Pathology, Ecology, Statistics, Epidemiology, Immunology, Microbiology, and a large part of the clinical staff. The Children's Hospital clinical staff and Research Institute, the Los Angeles Medical Society, the Los Angeles Demography Human and Animal Health Departments are all cooperating to a remarkable extent. Currently the Cancer Surveillance Program (CSP) includes participation of 83 hospitals furnishing 70% of the 30,000 hospital beds in Los Angeles County and City. Within a few months all of the hospitals with 100 or more beds will be fully participating. The CSP is designed by the end of FY 1973 to furnish within 3 weeks after histological diagnosis necessary information on all of the 20,000 cancer cases estimated to occur in Los Angeles, thus providing a "now" registry of still living cancer patients, many of whom will be available for contemporary epidemiological and etiological studies.

Specific epidemiological studies of human cancer.

Breast cancer: The purpose of this study is to find out if daughters of mammary tumor cases (and controls) have type B virus particles in their milk, and thus to determine if the human, like mouse, transmits mammary tumor virus in mother's milk. The cancer surveillance program has already provided a list of 247 living mammary cancer patients under age 60, with permission by physicians and patients to interview 129 having already been received; refusal rates run only 10% thus far. To date 41 milk specimens from daughters of cancer patients have been collected. Specimens from controls (no history of mammary cancer) are also being collected. These will be tested blindly for RDP and B particles as described above (VII). The specimen collections are being supplemented by an epidemiological survey based on historical family tree analyses for evidence of predisposition to cancer in general.

Hodgkin's disease: The relation of this disease to the following factors are under study: (1) tonsillectomy and appendectomy; (2) EB virus; (3) RNA tumor viruses; (4) HLA antigen phenotype; and (5) case clustering (as in Albany). The cancer surveillance program provides the case and control patients for contemporary study.

Preliminary observations have delineated apparent clusters in heroin addicts; five cases attended one junior high school. Tests by the Henle's of 40 cases showed 11 with antibody titers to EB virus of 1 to 160 or greater; the significance of this observation can only be clarified by further studies.

Green's very recent evidence of high levels of homology between RD114 type C RNA $\rightarrow$ DNA with RNA from Hodgkin's cases provides an exciting new area for molecular studies of tumor cells of Hodgkin's cases and tumors from other types of malignant lymphoma.

Young genital tract cancer: The USC surveillance group (headed up by Dr. Henderson) in cooperation with George Linden of the California Tumor Registry confirmed previous reports of increased vaginal cancers in the 10-19-year-old group; however, they also found an increase in cancer of the testis. Histories in the Los Angeles vaginal cancer patients indicate in utero exposure to maternal treatment with stilbesterol. Very preliminary findings suggest that bladder cancer may also be increased by this previously common treatment for threatened abortion.

Composition of airborne particles (smog) in 4 Los Angeles locations: The carcinogenic activity of Los Angeles smog extracts (in benzene and methanol) reported by Freeman and Rhim (see IV above) provided the impetus to analyze and fractionate the differing smogs available in 4 different areas of Los Angeles County. Benzene fractionations from three areas, while quantitatively different, were similar in composition, reflecting almost exclusively automobile emissions. The local area included other emissions from chemical plants and oil refineries. In one of the 4 areas, metals (iron, nickel, cobalt) were elevated, presumably due to iron smelting. Recently derived methanol extract fractions which were shown to be oncogenic by Rhim and Freeman contained large amounts of ammonia nitrate and will be studied further. Drs. Gordon and Bryan who are in charge of the Los Angeles smog collection and analysis have established valuable cooperative arrangements with the local and state programs concerned with environmental pollution, including air quality.

Natural history of cancer and type C and B RNA viruses in wild mice: Underway since 1969, this study has now provided valuable information on the natural cancers and RNA cancer virus expressions in a natural species. As in most other natural species, most populations of wild (feral) mice were found to be relatively free of type C RNA tumor virus expression during early and middle life (up to 20 months). The onset of almost all cancer was delayed until after 24 months of age; peaks of tumor incidences occurred at 27-29 months and at 33-40 months. In 7,000 mice observed, only 61 spontaneous tumors were observed at death--23 lymphomas, 8 sarcomas, 26 adenomas of the lung; 5 miscellaneous. Type C particles were observed by electron microscopy in 5 of 7 lymphomas, 0 of 8 sarcomas. Despite hundreds of attempts, only one mouse from these populations yielded infectious RNA tumor virus; this virus had all the properties of the type C viruses of laboratory mice except that the envelope was distinct from the Gross-like virus found in laboratory mice. Recently a new isolated population of wild mice was discovered (Lake Casitas area): 80% reveal infectious type C virus. Type B virus has been seen in about one third of the mammary gland tissues examined; however, these mice were not used for breeding after trapping, which probably accounts for the lack of mammary cancers in the geriatric females.

Natural history studies of type C RNA virus in cats with lymphoma, other cancers and anemia: In cats with cancer and/or anemia, gs antigen and type C particles were found to be quite common.

	gs antigen		type C particles	
Lymphomas	23/32	72%	26/37	70%
Sarcomas	1/4	25%	2/16	13%
Carcinomas	0/25	0%	10/37	37%
Anemia	9/10	90%	12/15	80%
Normal	2/20	10%	1/12	8%

In studies by Dr. Raymond Gilden of Flow Laboratories and Mr. Horace Turner of VCB, sera from over 80 cats with spontaneous or virus-induced lymphoma or sarcoma were all negative for antibody to species-specific gs antigen in CF and RIPI tests, thus confirming previous studies suggesting that cats are immunologically tolerant for this antigen.

An epizootic study of solid tumors among chickens: During October, 1971 increased numbers of solid tumors (fibrosarcomas, hemangiomas, nephroblastomas) were observed in 8-week-old chickens being processed as fryers in a USDA inspected packing plant located in Los Angeles. Such tumors had been rare to absent prior to the outbreak which followed the institution of the use of Marek's disease vaccines at birth. However, since some unvaccinated birds held as controls also revealed fairly high incidences of solid tumors, the role of the vaccine was not clear. However, virtually all of the solid tumors tested were positive for gs antigen at high levels. Studies by Vogt and Weiss of USC (also supported by STS funds) resulted in the isolation of both avian RNA tumor viruses and herpes viruses. As expected from previous VCB studies, no sarcoma-inducing viruses were recovered from the sarcomas. Interestingly, tests of typical Marek's disease lesions showed nearly 100% to be positive for type C RNA gs antigens. These findings suggest that natural Marek's disease virus and the vaccine (attenuated live turkey Marek's-like herpes virus) may in some fashion increase the virogene and oncogene expressions of type C RNA virus, thus leading to the solid tumors characteristically produced by the avian RNA tumor viruses. This study will continue during the next fiscal year in cooperation with the Los Angeles chicken industry and the USDA inspection department.

Additional Important New Findings and Developments

Preparation of molecularly purified species-specific gs antigens: Drs. Oroszlan and Gilden have now prepared highly purified (electrofocused) species-specific gs antigens and antisera in guinea pigs for the RNA tumor viruses of the following species: mouse, cat, rat, hamster, human? (RD114), viper and chicken. They are each species-specific in gel diffusion, radioimmune precipitation inhibition, and complement-fixation. Gilden, Parks,

Riggs, Turner, Huebner and Henderson are using these reagents for monospecific antigens and antisera in extensive searches for additional expressions of these subinfectious but specific expressions of RNA tumor viruses in the natural species. Such surveys have led to discoveries of infectious RNA tumor viruses in wild (feral) mice, cats and chickens with spontaneous tumors. Similar surveys for specific virus expressions are now planned in numerous human and other primate cancers, using antisera to specific gs and envelopes of the RD114, woolly monkey and gibbon ape virus - the latter in cooperation with Drs. Bustad, Kawakami and others working on the STS-SVCP contract at the University of California, Davis.

Discovery of woolly monkey and gibbon ape viruses: Drs. Theilen and Kawakami and associates at the University of California, Davis, reported isolations of viruses having the characteristic properties of type C RNA viruses from a sarcoma of a woolly monkey (SSV) and from a lymphosarcoma of a gibbon ape (SLV). Since both of these viruses can be grown in significant amounts in a number of primate cell lines, it should be possible soon to produce virus-specific gs and other viral antigens and antisera which should prove invaluable for doing natural history studies of virus and cancer incidences in these two species. Similarly, the species-specific and interspecies reagents should make it possible to determine possible cross reactions amongst the primate viruses. Of course the demonstration of type C RNA viruses in two primates increases the likelihood that the virus genome will also be found in humans.

Activation of EBV virus in virus-free Burkitt tumor (Raji) cells: Dr. Berge Hampar (VCB) and his associates reported activation of EBV virus in the EBV-free Raji strain of Burkitt's lymphoma with the use of BrdU, a finding confirmed by Dr. Paul Gerber of DBS. Using the same technique, Hampar also activated EBV virus in the Levene lymphoblastic cells recovered from pleural fluid. This procedure should make it possible to screen large numbers of lymphoblastic leukemia cell cultures for covert EBV virus. It may also help determine whether all virus-free Burkitt cell clones contain the genome.

Activation of type C RNA tumor virus expressions in cells transformed by polyoma virus: One of the major unanswered questions in viral transformation by the "oncogenic" DNA tumor viruses (polyoma, SV40, adenoviruses, and herpes type viruses) is the source of the transforming oncogene. Are the oncogenic genes responsible for cell transformation by the DNA viruses part of the viral DNA or the cell DNA; and further, does the endogenous RNA tumor virus genome participate in the transformation process? Recent findings by Drs. Freeman, Kelloff, Rhim, Huebner, and Mr. Lane indicate that like chemical carcinogens (see III above), polyoma and SV40 viruses do interact with RNA tumor virus genomes. Tumors and transformed cells in NIH Swiss mice and in hamsters induced by polyoma virus reveal large amounts of species-specific gs antigen when the cells are transplanted subcutaneously in newborns of these respective species. The local hamster tumors have infectious HaLV as well as gs antigen; neighboring and other tissues (spleen, thymus, etc.) are negative for antigen and virus. When polyoma-transformed NIH Swiss

cells were transplanted into newborn Swiss mice the tumors were strongly positive for gs antigen but negative for infectious virus. Since the normal hamster and NIH Swiss mouse otherwise are negative for infectious viruses, and the latter apparently cannot under any conditions make infectious virus (Rowe, Hartley and Huebner), the evidence favors amplification in the tumor of RNA virus expressions induced by the polyoma virus. Since polyoma, SV40 and adenoviruses have not been shown to cause tumors in their natural hosts (extensive data are universally negative) we interpret these findings as favoring the hypothesis that neoplastic changes produced in heterologous cells and animals by these viruses are due to derepression or activation of the RNA virus genome.

Temperature sensitive mutants of avian sarcoma and polyoma viruses: Conditional lethal (ts) mutants of tumor viruses represent one of the tools for measuring the specific activities of viral genes in virus replication and neoplastic transformation or tumor induction. Walter Eckhart of the Salk Institute, in producing a number of genetically stable mutants of polyoma virus, has been able to show the importance of polyoma viral gene determinants in maintaining the transformed state and also for T antigen expressions. Similarly, Peter Vogt and his associates at USC have established a number of mutants of Rous sarcoma virus. With Dr. Max Burger he has shown that when a Rous virus mutant was shifted from permissive to the nonpermissive temperature, the cells lost much of their agglutinability with jack bean agglutinins. When returned to permissive temperatures, the agglutinability returned to previous levels.

Recapitulation:

The first order of business we believe is to identify the nature and the origins of the cancer-inducing oncogenes in cells. During the past two years SVCP-supported and other scientists have gone a long way towards achieving this first objective, having demonstrated the validity of the RNA viral oncogene hypothesis (and perhaps to some extent the Temin protovirus hypothesis) in at least 4 distinct categories of vertebrate animals (mouse, rat, hamster and chicken). Other associates working with inbred mice and chickens have succeeded, with mendelian crossing and backcrossing experiments, in identifying host genes which regulate and therefore serve as determinants of expression of the inherited virogenes and oncogenes which make up the RNA tumor virus genome. Additional new information relates to inborn genetic defects of inbred animals which are known to predispose and predetermine high risks of many types of cancer; cancers quite similar to those which in man are also attributed to genetic defects.

It is a matter of record that the VCB-STS programs have assumed leadership in developing the technological tools needed for identification and quantitation and assays of natural and induced expressions of the genes of the RNA tumor viruses in normal and neoplastic cells.

We have also developed new very sensitive and rapid *in vitro* test systems for quantitative assay of carcinogenic chemicals found in increasing amounts

in human environments. In these tests we are measuring simultaneously in the several cell systems the carcinogenic transforming effects in cell cultures of (a) endogenous viral genomes and (b) added virus genomes and of the exogenous chemicals.

We are employing newly developed simplified but sensitive serological procedures in large scale natural history studies of natural expressions of RNA tumor viruses in human, feral mouse, cat, rat and chicken populations in natural ecologies with the purpose of determining the role of these virus expressions in the spontaneous cancers of these species.

Several projects in STS and VCB are engaged in (1) studies in cell cultures and inbred animals of natural and synthetic oncogene (and virogene) suppressing chemicals, and (2) studies of natural and induced immune responses that offer hope for immunological control of cancer.

Obviously, therefore, there is no way to avoid emphasizing the fact that the main focus of the VCB and STS research program is the study of the validity of the RNA viral oncogene hypothesis proposed by VCB scientists in 1969. The hypothesis agrees with most geneticists working in cancer that the genetic determinants (seeds) of the cancerous changes in a cell are part of natural gene inheritance; therefore these gene determinants (according to modern understanding of the genetic code) must be represented on the cellular DNA as polynucleotide sequences of all vertebrate cells. The hypothesis, however, further proposes that the information in all vertebrate cells has the capacity to make most or all of components (RNA $\rightarrow$ DNA and proteins) of the RNA tumor viruses, including the transcriptase and translation products required for oncogenic transformation. Of course, like many other cell genes, it is postulated that the RNA virus genome is properly controlled by host regulating gene systems in order to insure species survival. It is presumed that in an evolutionary context, infectious RNA (tumor) viruses must have provided certain advantages at one time in pre-vertebrate periods, but that now infectious expression and horizontal transmission (although occasionally observed) is no longer necessary for survival of the genome. This implies of course that horizontal transmissions of the RNA tumor virus genome in most higher species as a direct cause of cancer is likely to be rare or non-existent.

In higher vertebrates - in dogs, other domestic animals, primates and humans - where overt virus expression is rarely observed occurring spontaneously, expression of either the virogene or oncogene is viewed as the consequences of (a) genetic defects detected early in life, or (b) breakdowns of gene regulation and immune surveillance later in life due to environmental, mutagenic and/or oncogenic factors, or in many cases, simply senescence.

Finally, it is clear that the oncogene hypothesis and the concept of universal inheritance of the RNA tumor virus genome was not the result of brilliant intuition, but an inescapable conclusion forced on us by the overwhelming weight of extensive data, some of it old, but much of it contemporary, which revealed that the phenotypic expressions (often noninfectious) of the virus

in association with neoplastic state did not depend in nature on horizontal spread of infectious virus despite an occasional overt manifestation. When it was found that expressions of group-specific (gs) antigen expressions of the virus were commonly expressed in spontaneous and induced tumors and embryonic tissues of inbred mice, chickens and hamsters, most often in the total absence of infectious virus, it could only be concluded that the genome must be inherited.

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*Does not include publications on which first author is a member of another NCI Branch or a project director on a SVCP-supported contract (see Contract Publications.)

APPENDIX

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Dr. Bernard Talbot
Dr. Wade Parks
Dr. Gary Kelloff
Dr. Frank Portugal
Dr. Josephine Simonds
Dr. Bernice Moll
Dr. Paul Arnstein
Dr. Berge Hampar
Dr. Kenneth Rand
Dr. Padman Sarma
Dr. Victor Zeve

California State Dept. of Public Health (NIH-NCI-E-43-68-997)

Dr. Edwin Lennette
Dr. John Riggs
Dr. Lyndon Oshiro
Dr. D.O.N. Taylor

California State Dept. of Public Health (NIH-NCI-E-69-87)

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Dr. J. Michael Bishop
Dr. Warren Levinson
Dr. Leon Levintow
Dr. J. M. Taylor
Dr. A. J. Faras

Princeton University (NIH-NCI-E-71-2372)

Dr. Max Burger

University of California, Berkeley (NIH-NCI-E-71-2173)

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Dr. T. Gurney
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Dr. Richard Griesemer
Dr. Thomas Kawakami
Dr. JaRue Manning
Dr. Charles Aldrich
Dr. Patricia Buckley
Dr. Donald Dungworth
Dr. Sally Huff
Dr. Niels Pederson
Dr. David Slausen

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Flow Laboratories, Inc. (NIH-NCI-E-71-2097)

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Dr. J. Derge
Dr. E. Hutcheson
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Dr. Hans Meier
Dr. Benjamin Taylor
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Dr. Marianna Cherry
Dr. Hans Heiniger

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Dr. J. Poiley
Dr. J. Woo
Dr. Carrie Whitmire
Dr. R. Salerno
Dr. R. Kouri
Dr. M. Vernon
Dr. M. Elder
Dr. J. Parker

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(NIH-NCI-E-72-2032)

Dr. Peter Vogt
Dr. Robert Friis
Dr. Robin Weiss
Dr. Donald Blair
Dr. Maxine Linial
Dr. William Mason
Dr. John Wyke

St. Louis University
(NIH-NCI-E-43-67-692)

Dr. Maurice Green
Dr. Henry Pinterton
Dr. Kei Fujinaga
Dr. Nobuyuki Takemori
Dr. Heschel Raskas
Dr. Helen Thornton
Dr. Domenica Attardi
Dr. Koichiro Shimada
Dr. M. C. Loni
Dr. Miriam Margalith
Dr. Corrado Gurgo
Dr. Nobuo Tsuchida
Dr. Dean Tsuei
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Dr. Samuel Salzberg
Dr. Gary Gerard
Dr. Hyune-Mo Rho
Dr. G. Shanmugam
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Salk Institute
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Dr. Murray Gardner
Dr. Hugh Edmondson
Dr. J. Parker
Dr. J. Lukes
Dr. Robert M. McAllister
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Dr. Brian Henderson
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Dr. B. Pal
Dr. E. Officer
Dr. R. Gordon
Dr. R. Bryan
Dr. H. Charman
Dr. E. Johnson
Dr. S. Rasheed

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Dr. Leonard Hayflick
Dr. King Tam
Dr. E. Stanbridge

Weizmann Institute
(NIH-NCI-E-69-2014)

Dr. Leo Sachs

Wistar Institute
(NIH-NCI-E-71-2092)

Dr. Anthony Girardi

Twenty-five scientific publications resulted from studies carried out in this Branch during this year.

Serial Number NCI 4922
1. Viral Carcinogenesis Branch
OASDVO, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Laboratory and Field Studies of the Role of Type C RNA Viruses in Human and Animal Cancer

Previous Serial Number: Same

Principal Investigators:
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Dr. Bernard Talbot
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Dr. Robert Peters, Microbiological Associates, Inc.
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Dr. Gerald Spahn, Microbiological Associates, Inc.
Dr. Louise Rabstein, Microbiological Associates, Inc.
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Dr. Eugene Zimmerman, Microbiological Associates, Inc.
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Dr. Ronald Salerno, Microbiological Associates, Inc.
Dr. John Parker, Microbiological Associates, Inc.
Dr. Raymond Gilden, Flow Laboratories, Inc.
Dr. Masakazu Hatanaka, Flow Laboratories, Inc.
Dr. Stephen Oroszlan, Flow Laboratories, Inc.
Dr. Cedric Long, Flow Laboratories, Inc.
Dr. Hans Meier, The Jackson Laboratory
Dr. Murray Gardner, University of Southern California
Dr. Brian Henderson, University of Southern California
Dr. Robert Bryan, University of Southern California
Dr. Robert Gordon, University of Southern California
Dr. Earle Officer, University of Southern California
Dr. Howard Charman, University of Southern California
Dr. P. Roy-Burman, University of Southern California
Dr. Suraiya Rasheed, University of Southern California
Dr. Robert McAllister, Children's Hospital of L.A.
Dr. Vaclav Klement, Children's Hospital of L.A.
Dr. Margery Nicolson, Children's Hospital of L.A.
Dr. W. Nelson-Rees, Naval Biomedical Research Lab.
Dr. Adeline Hackett, Naval Biomedical Research Lab.
Dr. Edwin Lennette, California State Dept. Public Hlth.
Dr. John Riggs, California State Dept. Public Hlth.
Dr. Lyndon Oshiro, California State Dept. Public Hlth.
Dr. Leonard Hayflick, Stanford University
Dr. Michael Bishop, University of California, San Francisco
Dr. Gordon Tomkins, University of California, San Francisco
Dr. Peter Duesberg, University of California, Berkeley
Dr. Leo Bustad, University of California, Davis
Dr. Thomas Kawakami, University of California, Davis

Project Description

Refer to Summary, Viral Carcinogenesis Branch, NCI

Honors and Awards: (Dr. Robert J. Huebner)

Guido Lenghi Award, National Academy of the Lincei, Rome, Italy, 1971

Publications:

Huebner, R.J., Sarma, P.S., Kelloff, G.J., Gilden, R.V., Meier, H., Myers, D.D. and Peters, R.L.: Immunological tolerance to RNA tumor virus genome expressions: significance of tolerance and prenatal expressions in embryogenesis and tumorigenesis. Ann. N.Y. Acad. Sci. 181: 246-271, 1971.

Huebner, R.J., and Gilden, R.V.: Inherited RNA viral genomes (virogenes and oncogenes) in the etiology of cancer. In Emmelot, P. and Bentvelzen, P., (Eds.): RNA viruses and host genome in oncogenesis. Amsterdam, North Holland Publishing Co., in press.

Huebner, R.J., Freeman, A.E., Whitmire, C.E., Price, P.J., Rhim, J.S., Kelloff, G.J., Gilden, R.V., and Meier, H.: Endogenous and exogenous RNA tumor virus genomes in chemical carcinogenesis. Monograph, Environment and Cancer, Collection of papers presented at 24th Annual Symposium on Fundamental Cancer Research, Houston, 1971, in press.

Serial No. NCI 4924
1. Viral Carcinogenesis Branch
   OASDVO, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: (A) Studies on the Natural Expression of HaLV in Normal Animals and In Vivo and In Vitro Studies of the Effect of Chemical Carcinogens and Mutagens on HaLV Expression.

(B) Production of Antisera to the Envelope, gs-1 and gs-3 Antigens of the Mammalian C-type Viruses.

(C) In Vitro Studies of Host Cell Gene Control of C-type Viruses.

(D) Production of Synkaryons for Use in Mammalian C-type Virus Studies.

(E) Immunological Comparisons of Murine Sarcoma Viruses Grown in Mouse and Human Cells.

Previous Serial Number: Same

Principal Investigator: Dr. Gary J. Kelloff

Other Investigators:
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Mr. William T. Lane, VCB, NCI
Mr. Worth I. Capps, VCB, NCI

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Dr. Stephen Oroszlan, Flow Laboratories, Inc., Rockville, Maryland
Dr. Cedric Long, Flow Laboratories, Inc., Rockville, Maryland
Dr. Aaron Freeman, Microbiological Associates, Inc., Bethesda, Maryland
Dr. Hans Meier, The Jackson Laboratory, Bar Harbor, Maine

Man Years:

Total:	7.0
Professional:	2.0
Other:	5.0

Project Description

Objectives:

(A) (1) Study of the prevalence of HaLV in normal hamsters: (2) *In vivo* studies on co-carcinogenesis of the hamster leukemia virus HaLV and chemical carcinogens: (3) *In vitro* studies on the activation of HaLV by BrdU and other carcinogens.

(B) (1) Production and characterization of neutralizing antibody to the mammalian type C viruses: (2) Production and purification of gs antigen of mouse and hamster type C viruses and preparation of gs antisera to these antigens: (3) Preparation of interspecies specific antisera (anti gs-3): (4) Fluorescent antibody studies of mammalian type C virus gs antigens.

(C) *In vitro* studies of host cell gene control of virus expression.

(D) (1) Production and characterization of synkaryons to examine the possibility of derepression of latent virus: (2) Establishment of synkaryons of several species in search of marker chromosomes needed for type C virus replication: (3) Study of the metabolic requirements of the rescue process of MSV from HT-1 cells.

(E) (1) To study and characterize the immunological and virological properties of the Kirsten strain of murine sarcoma virus (KiMSV) grown on mouse cells to KiMSV grown on human cells - as a model for study of envelope antigen changes in host range changes.

Methods Employed and Major Findings:

(A) Standard tissue culture methods for virus growth and titration were used. Antisera has been prepared against the purified gs antigen of the hamster type C viruses (Oroszlan *et al.*). Testing consisted of complement-fixation and fluorescent microscopy. Antigens for CF were 20% extracts of tissues from normal and tumor-bearing hamsters and from *in vitro* preparations. Fluorescent microscopy was done by the indirect method using fluorescein conjugated anti-guinea pig gamma globulin. Screening of tissues were also performed by electron microscopic examination. H^3U labeling techniques of virus and RNA dependent DNA polymerase assays were performed in the BrdU experiments.

In studying the normal expression of HaLV in normal hamsters, tissues from normal uninoculated hamsters of the NIH, LSH and Graffi strains were screened in complement-fixation for the presence of HaLV gs antigen. Animals ranging in age from one month to twenty-five months were sacrificed and antigen preparations of various organs were tested. Without exception group-specific antigen was not detectable in any of these tissues from fifty-five animals. Electron microscopic examination and direct isolation attempts of these tissues also did not yield any evidence of type C virus. Earlier studies of type C virus expression in the mouse suggested that embryonic tissues would

be a likely place to detect the endogenous hamster virus. From the three strains of hamsters, 8-9-day-old embryos and organ pools from 14-15-day-old embryos were examined. Unlike the postnatal tissues from the Graffi hamsters, the 8-9-day-old Graffi embryos were convincingly positive in CF. Of 40 whole embryos tested from 7 litters, 24 were found to be positive in complement-fixation at antigen dilutions ranging from 1:4 to 1:16. All but one litter had at least one positive embryo, however. Repeated isolation attempts from preparations of these whole embryos, of embryonic organs, of placentas and gravid uteri, have failed to yield evidence of virus, as has electron microscopic examination. Virus isolation attempts by rescue techniques are in pregress. Studies of hamster tumors induced directly by chemical carcinogens--3 methylcholanthrene and 7,12-dimethylbenzanthracene--have shown a significant percentage of these tumors to be positive for HaLV. In those tumors that were not positive by direct induction by the chemical, transplant passages of the same tumors generally became positive. The same summary statement can be used to describe hamster tumors induced by oncogenic DNA viruses--DNA and polyoma. Of interest is that in these animals with HaLV positive tumors, the spleens from the same animals have never shown evidence of HaLV. Also, in studies on the LSH strain of hamster, although 3MC and DMBA are equally effective in inducing tumors, no DMBA induced tumors were found to have HaLV, whereas many of those induced by 3MC were positive.

Treatment of HT-1 cells with BrdU has been shown to result in activation of the gs antigen of HaLV. These studies are in progress.

(B) For production of neutralizing antisera, continuous cell lines shedding AKR, Rauscher leukemia virus (RLV), HaLV and FeLV have been produced. From supernatant fluids, virus was banded on sucrose density gradients and was used for immunization of rabbits. Acutely infected secondary embryo fibroblast cultures have been used to produce virus for gs antigen purification and immunization. Although these studies have just been initiated, the yield of virus in the supernatant fluid appears to be quite good. Standard tissue culture methods have been used and virus neutralization tests have been done by the focus induction and plaque induction techniques. Hyperimmunization of rats with homogenates of MSV tumors as described by Gilden et al. has been initiated for production of interspec (gs-3) antibody. The gs antigens of these species of type C virus described above will be used in gel diffusion and complement-fixation to characterize these reagents.

The availability of high titered neutralizing antisera has allowed us to do classical virological experiments which require significant quantities of these reagents. We have obtained considerable quantities of sera with neutralizing titers in excess of 1:160 and, for example, this has been used in the media of infected cultures to prevent virus spread and allow conclusions to be made about virus rescue in cells that have been fused. In collaboration with Dr. Hampar these reagents have been used when conjugated with ferritin to examine the envelope antigen of the type C viruses by E.M. Attempts to make gs antigen and anti-gs antisera have thus far been limited

to virus production and sucrose banding of virus. Hyperimmunization of rats with tumor homogenates of MSV is in progress and initial test bleeds have indicated a reactivity of these sera with ether-treated preparations of banded RLV and FeLV in a small percentage of animals.

Cells infected with mouse, hamster and cat leukemia viruses have been examined by fluorescent microscopy with anti-gs-1 and anti-gs-3 antisera. The species specificity of the gs-1 antigens and the sharing of the gs-3 on the same molecule as reported by Gilden et al. has been confirmed. These studies also demonstrated the efficacy of the use of FA in studying the gs antigens with sera made to the purified antigens. These techniques will be useful and implemented in studies where a small population of cells might be gs antigen positive (e.g., BrdU experiments).

(C) The studies of host cell gene control of virus expression have just been initiated in collaboration with Dr. Hans Meier. We have recently obtained his four inbred lines of AKR and C57L combinations characterized by their spleen antigen phenotypes and are currently making cell stocks from embryonic tissues of these mice. We have developed proficiency in performing XC plaque assays for titration of MuLV.

(D) The methods employed in synkaryon formation have been described. HT-1 cells have been made thymidine kinase-negative by prolonged passage in BrdU (6 months). BALB/c cells have been made H6PRT-negative by passage in thioguanine. The selection system then involved HAT medium (hypoxanthosine, aminopterin and thymidine) which, when these two cells are fused, allows only the synkaryons to survive. Similar selective systems were used for production of synkaryons of HT-1 cells and human leukemia lymphocytes. Other methods involved the measurement of the drug-treated individual lines (or the enzymes that they had been made deficient in), chromosome examination, iso-electrofocusing of enzymes from the synkaryons to identify them as to their species of origin by their isoelectric points (the final proof of a true synkaryon). Classic virologic challenge and assay studies, using the focus assay and COMUL tests as well as H^3U gradient banding procedures, polymerase assays and electron microscopic examination, have been utilized. Sendai-inactivated by β-propriolactone has been used for the fusion studies. Metabolic inhibitors such as cytosine arabinoside, actinomycin D, and puromycin have been used, as has the uptake of H^3T and H^3U and H^3AA into acid insoluble (TCA) material, to study the inhibition of DNA, RNA and protein synthesis.

Synkaryons of hamster-mouse cells (HT-1 C B/c 3T6) have been made (35 clones) and examined for derepression of MuLV by direct isolation attempts, CF, E.M., H^3U labeling and polymerase assays, and no activation of the endogenous MuLV was detectable. The same experiments have been done with HT-1 X human leukemic lymphocytes with no evidence of virus activation. These hybrid clones have all been shown to contain the rescuable defective MSV genome contained in the parent HT-1 cell. Likewise, BrdU treatment of these hybrid cells has not resulted in virus activation using the above described parameters. These cells have been conclusively shown to be hybrid cells by

enzyme analysis and virus host range analysis. Heterokaryons produced between HT-1 X B/c 3T6(RLV) - (infected with RLV) - have been shown to produce leukemia and sarcoma virus with different clones having different quantitative expressions of these two viruses. Since heterokaryons are unstable and randomly lose chromosomes, the question of host cell genetic control of virus expression can be systematically studied in these cells. This requires extensive chromosomal analysis, which has not as yet been done.

The use of Sendai mediated cell fusion has been exploited in rescue studies of the MSV genome by fusing HT-1 cells with cells shedding mouse leukemia virus. By this fusion rescue technique, rescue was noted to occur within two hours after fusion. This short time interval for rescue to occur suggested that the metabolic requirement for rescue could be systematically studied. That is, can one "shut off" DNA, RNA, or protein synthesis, immediately fuse cells, and observe rescue to take place? Theoretically, if the defective MSV genome exists as multiple RNA copies in the HT-1 cytoplasm (reported by Green) and if BALB/c cells shedding RLV contain multiple copies of unassembled RLV coat antigens, one ought to be able to demonstrate rescue of the defective MSV genome in the absence of DNA, RNA or protein synthesis (or in the absence of one of them). Extensive experiments have demonstrated that de novo synthesis of all of these macromolecules appears to be necessary before rescue can take place. However the sensitivity of the rescue process on DNA dependent RNA synthesis (as demonstrated by actinomycin D) seems to be much greater than the other metabolic requirements, suggesting that if the defective genome does exist in RNA form in HT-1 cells, it has such a high turnover rate that de novo synthesis DNA $\longrightarrow$ RNA is required before rescue can be effected.

(E) Continuous cell lines shedding KiMSV/3T3 and KiMSV/909 have been derived respectively from cloned foci of BALB/c 3T3 cells and a human skin fibroblast line. Neutralization tests by focus reduction has been quantitatively and extensively done. Radioactive labeling with H^3uridine, C^{14} amino acid, and C^{14} glucosamine has been done to compare quantitatively the differences in host range of KiMSV/3T3 and KiMSV/909. Banded preparations of the two viruses were made and quantitated by protein determination and CF, using antisera to the murine gs antigen. Viral preparations of KiMSV/3T3 with infectivity titers of 10^5 on homologous cells (B/c 3T3) showed no activity on heterologous cells (909). Conversely, preparations of KiMSV/909 with infectivity titers of 10^3 on 909 were not active on B/c 3T3 cells. Neutralizing antisera to the agents have been produced and the neutralization endpoints of the two sera show a definite cross neutralization in both directions with about 8 units of difference from homologous to heterologous virus. If complement is added to the neutralization reaction, the neutralizing endpoints of the heterologous reaction become equal, the homologous reaction suggesting that the two viruses show major antigens but are masked by cell membrane. Interestingly, antisera to normal host cells with complement will neutralize the virus grown in those host cells but not that virus grown in the heterologous host. Experiments labeling the two viruses with C^{14}AA and subsequent analysis by acrylamide gel electrophoresis shows that the two viruses have identical

resolvable protein subunit structures. Initial studies of the same type using C^{14} glucosamine label also suggest that the glucosamine-containing structures of the two viruses also have properties indistinguishable from each other.

Significance to Biomedical Research and to the Program of the Institute:

(A) The type C virus expression in the hamster remains the best characterized animal model system that most resembles the presumed situation in man. Throughout the normal animal's life span, with few or no exceptions, HaLV cannot be demonstrated; however in tumors induced by unrelated sarcoma viruses and chemicals, HaLV is readily isolatable. Although one cannot rigorously rule out that HaLV is merely a passenger in these tumors "seeded" from a source elsewhere in the hamster, there are several findings that suggest this is not true. There is no HaLV isolatable from normal animals, there is no HaLV detectable in the spleens of animals with positive tumors, there is a differential expression of HaLV in tumors induced by chemicals which are equal in their oncogenic potential, and preliminary observations show that BrdU treated HT-1 cells appear to have a much greater frequency of HaLV expression when producing tumors in the animal than normal HT-1 cells. Quantitative experiments are in progress to examine this last mentioned phenomenon in attempts to resolve this question. There is unequivocal evidence that hamster cells seem to exert a greater control (genetic?) of complete infectious type C virus expression than other species. Cell packs of hamster cells and mouse cells infected with their respective type C viruses can be shown to have equal titers of homologous gs antigen by complement-fixation, yet yield studies of infectious virus, H^3U labeled peaks and polymerase assays of the supernatant fluids from these cells consistently show about 10-fold (1 log) more mouse virus than hamster virus. Fluorescent microscopic observation of such cells consistently show an even greater percentage of gs antigen-positive hamster cells than mouse cells. These findings suggest that the hamster cell exerts a genetic control of infectious HaLV expression.

(B) With finding of BrdU activation of expression of part or all of the endogenous virogenes, the importance of the availability of large quantities of antisera to all of the known viral antigens cannot be overemphasized. The sera will be used in complement-fixation, immunodiffusion, and fluorescent antibody studies. Although antisera specifically to envelope antigens is not yet available, it should be producible using published avian tumor virus model systems. The neutralizing sera that we have made should be valuable in screening for this purification.

(C) With the findings that natural expression of type C virus in human and many other species is minimal, and with the evidence that type C RNA viruses probably have a DNA intermediate, studies of host cell gene control of virus expression are essential.

(D) It has been found that endogenous type C virus could not be derepressed in heterokaryons carrying the defective MSV genome with or without BrdU

treatment. These experiments have made available cell substrates which should be useful to isolate new human virus candidates, as the human X hamster hybrids carry MSV and progeny virus can be shown to have focus forming activity. With the availability of new techniques for specifically staining chromosomes and with the random (but somewhat predictable) loss of chromosomes in heterokaryons, a systematic long range approach to the study of what host cell genes control viral expression can be done on such hybrids. These studies would require starting anew and making new hybrids, and since we have priorities which supersede these studies, they probably will not be initiated, at least not in the near future. The requirement for de novo RNA synthesis for rescue is interesting, but cannot be further exploited experimentally.

(E) These studies emphasize the caution which must be exercised in interpreting observed viral changes when passaged from one host to the other as being due to genetic changes, as opposed to mere phenotypic changes. The finding of equivalent neutralization titers of sera to homologous and heterologous viruses with the use of complement suggest that the observed changes in host range of KiMSV passaged on different cells is due merely to phenotypic host cell modification of the virus. Even with glucosamine labeling studies this is hard to prove conclusively, especially if you can't get the virus to cross infect the cells that the heterologous agent is growing in.

Proposed Course:

(A), (B) and (C) - continuation of studies outlined above. (D) - use of hybrid cells that have been produced for selected specialized studies with a discontinuation of the major effort: publication of current findings. (E) - publication of current findings.

Honors and Awards:

None.

Publications:

Kelloff, G., Huebner, R.J., and Gilden, R.V.: Isolation and characterization of the hamster C-type viruses. Proceedings of the Vth International Symposium on Comparative Leukemia Research, Padova, Italy, 1971, in press.

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Kelloff, G., Hatanaka, M., and Gilden, R.V.: Assay of C-type virus infectivity by measurement of RNA dependent DNA polymerase activity. Virology, in press (April 1972).

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Serial No. NCI 4925
1. Viral Carcinogenesis Branch
   OASDVO, Etiology
2. Ecology and Epizoology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies of C-type RNA Tumor Viruses

Previous Serial Number: Same

Principal Investigator: Dr. Padman S. Sarma

Other Investigators: Dr. Robert J. Huebner, VCB, NCI
Dr. Paul Arnstein, VCB, NCI
Mr. Horace C. Turner, VCB, NCI

Cooperating Units: Inside NIH:
Dr. Adi Gazdar, VLLB, NCI

Outside NIH:
Dr. M. Gardner, USC School of Medicine, Los Angeles, California
Dr. R. McAllister and Dr. V. Klement, Children's Hospital, Los Angeles, California
Dr. R. Gilden, Dr. M. Hatanaka and Dr. S. Oroszlan, Flow Laboratories, Inc., Rockville, Maryland
Dr. L. Vernon, Microbiological Associates, Inc., Bethesda, Maryland
Dr. David Allen, Massachusetts General Hospital, Boston, Massachusetts

Man Years:

Total:	9.0
Professional:	2.0
Other:	7.0

Project Description

Objectives:

This project is concerned with basic studies on the isolation and characterization of the biologic, antigenic, biophysical and biochemical properties of known model type C RNA tumor viruses and those newly isolated from primate species. A large portion of the effort is directed towards the

development and use of sensitive in vitro systems for studying the prevalence and behavior of overt as well as covert type C viruses, utilizing the methodology developed in large part in this laboratory, such as, rescue of defective endogenous tumor virus genomes by trans-species rescue, in vitro co-cultivation technique, detection of noncytopathogenic type C viruses by CF antigen induction test, viral interference test, etc. Various approaches are being intensively applied to animal and human tumor systems having covert ("switched off") viral genomes in efforts to "turn on" or rescue overt expressions of the viral genomes.

Methods Employed and Major Findings:

(A) Studies on a human candidate type C virus. The RD114 virus, derived from a cell line of human rhabdomyosarcoma, was characterized. These studies were done in collaboration with investigators of the University of Southern California (McAllister, Gardner), Flow Laboratories (Gilden, Hatanaka, Oroszlan), and Microbiological Associates (Kabigting, Vernon).

Our initial studies established important biologic and antigenic characteristics of this virus. The virus was shown to be infectious for human and feline embryo cultures. The virus was not found to contain the viral envelope antigens of the known feline leukemia viruses (subgroups A, B and C) or the species-specific group-specific antigen (gs-1) of the type C viruses of cat, mouse, hamster, rat or chicken. However, the virus contained the shared gs antigen of the mammalian type C viruses (gs-3 antigen) and a reverse transcriptase enzyme which suggested that the type C virus is of mammalian origin.

Virus growth in vitro was assayed by four procedures: (1) by the detection of the induction of the reverse transcriptase enzyme activity in the inoculated cultures; (2) by the detection of the incorporation of ^{3}H uridine into the virions released into the culture media of infected and labeled cultures; (3) by electron microscopic examination of thin sections of cultures; and (4) by a newly developed CF antigen induction test for RD114 virus, analogous to the COFAL and COMUL tests. The gs-1 antigen of the RD114 virus was detected in the infected cultures by CF test with an RD114 gs antiserum prepared in guinea pigs by Gilden and associates. The RD114 gs-1 antibodies failed to react with feline type C viral antigens.

Our recent studies indicated that although a variety of human cultures such as Hela, KB, HEp-2, are susceptible to infection with the RD114 virus, the cell most susceptible to infection is the parent line of RD cells (free of demonstrable virus) from which the virus producing RD114 was derived by cat passage. Whereas the titers in other human cultures were generally low ($<10^{3}$ per 0.2 ml), the titers in RD cells were in excess of 10^{4}. The availability of a sensitive culture for the RD114 virus has made it possible to perform virus neutralization tests for RD114 antibodies. Preliminary experiments showed that RD114 viral envelope antisera prepared in guinea pigs specifically neutralized 10^{3} infectious units of the RD114 virus, but not the same doses of the A, B and C serotypes of the feline leukemia viruses. The

absence of the gs-1 antigens of the various known lower mammalian type C viruses in the RD114 virus and the presence of the shared gs-3 antigen of the mammalian type C viruses, as well as the presence of a reverse transcriptase similar to the primate type C reverse transcriptase (experiments of Scolnick, Parks and associates) suggest that the RD114 virus may be of human origin.

(B) Activation of endogenous type C viruses by DNA analogs. Two cell lines of Rous sarcoma virus-transformed rat cells (XC cells, CCL-47) were found to release noninfectious type C viruses in low quantities. Treatment of the cultures with BrdU and IdU for 24 to 48 hours resulted in the release of a type C virus in high titers. The virus recovered was found to replicate poorly and only in NRK rat cells. The virus had the gs-1 antigen of the rat C-type virus as demonstrated by immunodiffusion tests with rat type C gs-1 antisera (collaboration with Dr. Gilden).

Similar chemical induction studies with human tumor cells such as RD and cultures derived from a Hodgkin's disease patient gave equivocal results. Although a reverse transcriptase enzyme activity was induced in these cultures, electron microscopy as well as other tests for production of noninfectious or infectious type C viruses gave negative results.

(C) Subgroup classification of the feline leukemia and sarcoma viruses. The viral envelope antigens of several strains of feline leukemia virus (FeLV) and feline sarcoma viruses (FSV) were characterized. The studies were performed by viral interference tests against the cell transforming effects of murine sarcoma virus with the viral envelopes of FeLV, and by viral neutralization tests. The occurrence of differential patterns of viral interference and the correlation of these findings with results of viral neutralization tests suggested that feline type C viruses contain distinct viral envelope antigens, similar to those described by Vogt and others, for the avian type C viruses. These findings enabled us to classify the feline type C viruses into three subgroups of A, B and C. The type-specific envelope antigen of the A subgroup viruses appeared to be shared by the B and C subgroup viruses as well. Subsequent virus cloning studies with or without the use of virus neutralizing antibodies established the fact that the presence of the viral envelope antigen of the A subgroup viruses in virus stocks of B and C viruses is due to the presence of an A subgroup virus as one of the components. Thus several field strains of feline leukemia viruses from the U.S.A. and Scotland, as well as established strains such as FL-74 (Theilen), and the three known strains of feline sarcoma viruses (Gardner, Theilen, McDonough) were all found to be virus mixtures.

The lack of cross interference and cross neutralization between cloned viruses suggest that the feline type C viruses gain entry into cells at cell receptor sites specific for each major viral envelope antigen. Thus virus penetration into cells is prevented either by the presence of an antigenically related virus at the specific cell receptor site (blockage to virus penetration) or by the inactivation of the antigen responsible for viral infectivity (cell penetration) by antiserum treatment.

(D) A new strain of murine sarcoma virus: biologic and antigenic interaction with the heterologous hamster host. The known strains of murine sarcoma viruses (MSV) fail to induce the mouse leukemia viral gs antigens or corresponding antibodies in the heterologous hamster host, despite the incorporation into the transformed hamster cells of rescuable MSV genome. On the other hand, we found that a newly described MSV, Gazdar MSV, induced mouse gs-1 antigen containing sarcomas in hamsters. Thus hamsters bearing primary or transplanted tumors consistently developed the mouse leukemia group-specific CF antibodies (collaborative studies with Dr. A. Gazdar). These hamster antisera were as useful as MSV rat sera for the detection and assay of murine leukemia gs viral antigens and the noncytopathogenic mouse leukemia viruses (the COMUL test). The induction of the gs antigen and corresponding antibodies appeared to be due to the presence in the hamster tumor preparations of a noninfectious type C virus with the biologic property of a sarcoma virus. Thus co-centrifugation of the virus with mouse leukemia virus resulted in a conversion of its property into an infectious murine sarcoma virus.

(E) Localization of the avian type C gs viral antigens in "leukosis free" chick embryos. The presence of viral gene products in most chick, as well as murine embryos, has suggested to some a role of leukosis viral genes in normal ontogeny as well as their implication in oncogenesis. As a first step in discerning their significance in the embryo, we attempted to determine the identity, concentration and distribution of the gs antigens in "RIF-free" chick embryos (collaborative studies with Dr. D. Allen, Massachusetts General Hospital). The presence of a specific gs antigen, gs-a, was identified in chick embryo cells and found largely confined to the microsome fraction. Electron microscopic examination of the "RIF-free" chick embryo cultures did not reveal abundant type C particles (noninfectious particles). Since the microsome fraction would also contain any C particles present, the high CF titers revealed suggests that much of the gs antigen is present in structures other than identifiable C particles. Hence the exact cellular locus and function of this viral gene product remains to be determined.

Significance to Biomedical Research and to the Program of the Institute:

Studies performed during the reporting period were directed in part towards getting definitive and much needed information on the delineation of the occurrence and characteristics of as yet undetected, as well as newly discovered, type C viruses of man, rat and mouse. These and other model tumor virus systems (cat and chicken) were intensively studied to better understand the biologic and antigenic similarities and interrelationships between the viruses of various species. These studies, as well as studies of the expression of the vertically transmitted endogenous tumor virus genomes, have been successful and provide tools for further studies of the natural occurrence and spread of leukemogenic and/or sarcomagenic viruses in homologous and heterologous species including man.

Progress was made in delineating the biological, biophysical, biochemical and antigenic properties of a virus, RD114, released by a cell line of human rhabdomyosarcoma cells. The possible human origin of this virus is currently under investigation.

Our previous studies showed that overt expression of the rat type C virus is very rare. Our present studies showed that type C rat viruses can be found in certain established cell lines of rat origin.

The studies of envelope antigenic relationships in feline type C viruses provided information on the occurrence of divergent serotypes. The studies permitted a subgroup classification of the feline type C viruses and gave valuable clues as to the mechanism of infection of feline cells by the feline type C viruses.

A new strain of MSV recently isolated from a NZW/NZB mouse was found to differ substantially from the presently known strains of MSV in its biologic and antigenic interaction with the heterologous hamster host. This study provided a practical method for the production of a highly useful research reagent in the hamster, the gs antiserum against the viruses of the murine leukemia sarcoma complex.

Studies on the prevalence and location of avian type C viral gs antigen in "RIF-free" chick embryos provided helpful leads for more intensive studies on the probable site(s) of cellular occurrence of the type C viral genome.

Proposed Course:

The recently developed CF antigen induction test for a human candidate virus, RD114, will be applied to detect the occurrence and behavior viruses having the gs-1 antigenic marker of the RD114 virus. Human, as well as cat sera, are being tested for the prevalence of naturally occurring envelope antibodies. Rescue techniques using helper viruses, helper cells and chemicals are intensively applied to uncover and characterize latent endogenous type C viral genomes of man and rat. The properties of simian type C viruses will be studied and compared with those of suspected human type C candidate viruses.

Honors and Awards:

None.

Publications:

Sarma, P.S. and Log, T.: Viral interference in feline leukemia-sarcoma complex. Virology 44: 352-358, 1971.

Sarma, P.S., Neubauer, R., and Rabstein, L.: Murine sarcoma and leukemia viral infection of mice. Effect of long-term treatment with Poly I·Poly C. Proc. Soc. Exp. Biol. Med. 137: 469-472, 1971.

Sarma, P.S., Gilden, R.V. and Huebner, R.J.: A complement-fixation test for the detection of viral antigens and infective virus of feline leukemia and sarcoma. J. Amer. Vet. Med. Ass. 158: 1055-1060, 1971.

Sarma, P.S., Huebner, R.J., Gilden, R.V., Baskar, J.F., and Gardner, M.B.: In vitro isolation and propagation of the GA strain of the feline sarcoma virus. Proc. Soc. Exp. Biol. Med. 137: 1333-1336, 1971.

Sarma, P.S., Log, T., and Theilen, G.: ST feline sarcoma virus propagation and cell transformation in vitro. Proc. Soc. Exp. Biol. Med. 137: 1444-1448, 1971.

Gazdar, A.F., Chopra, H.C., and Sarma, P.S.: Properties of a murine sarcoma virus isolated from a tumor arising in a NZW/NZB F_1 hybrid mouse. I. Isolation and pathology of tumors induced in rodents. Int. J. Cancer 9: 219-233, 1972.

Gazdar, A.F., Sarma, P.S., and Bassin, R.H.: Properties of a murine sarcoma virus isolated from a tumor arising in a NZW/NZB F_1 hybrid mouse. II. Physical and biological characteristics. Int. J. Cancer 9: 234-241, 1972.

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McAllister, R.M., Nicolson, M., Gardner, M.B., Rongey, R.W., Rasheed, S., Sarma, P.S., Huebner, R.J., Hatanaka, M., Oroszlan, S., Gilden, R.V., Kabigting, A., and Vernon, L.: C-type virus released from cultured human rhabdomyosarcoma cells. Nature New Biol. 235: 3-6, 1972.

Sarma, P.S. and Log, T.: Viral envelope antigens of feline leukemia and sarcoma virus. Bibl. Haematol., Karger (Basel/New York), in press.

Rabin, H., Theilen, G.H., Sarma, P.S., Dungworth, D.L., Nelson-Rees, W.A., and Cooper, R.W.: Tumor induction in squirrel monkeys by the ST strain of feline sarcoma virus. J. Nat. Cancer Inst., in press.

Allen, D.W., and Sarma, P.S.: Identification and localization of avian leukosis group-specific antigen within "leukosis-free" chick embryos. Virology, in press.

Serial No. NCI 4926
1. Viral Carcinogenesis Branch
OASDVO, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Survey of Naturally Occurring Oncogenic Viral Inhibitors.

Previous Serial Number: Same

Principal Investigator: Dr. F.H. Portugal

Other Investigators: Dr. Josephine Simonds
Dr. Bernice Moll

Cooperating Units: Inside NIH:

Dr. Robert J. Huebner, VCB, ET, NCI
Dr. Stuart A. Aaronson, VLLB, ET, NCI
Mr. William Lane, VCB, ET, NCI

Outside NIH:

Dr. G. Spahn, Microbiological Associates, Inc.

Man Years:

Total:	1.2
Professional:	1.0
Other:	0.2

Project Description

Objectives:

To determine whether natural inhibitors, which are capable of inhibiting viral induced carcinogenesis, can be isolated from cells infected with oncogenic virus. The basis of this study is the observation that such inhibitors (STAS) can be isolated from the supernatant media of certain infected mouse cell lines (Nature 227: 1021, 1970) using silicotungstic acid reagent.

Methods Employed:

Cell lines under investigation include $JLSV_5$, $JLSV_6$, $JLSV_9$, and $JLSV_{10}$. Preparation of inhibitor follows low speed centrifugation to remove cells. Polyethylene glycol is added to precipitate virus, followed by the addition

of silico tungstic acid to remove polyethylene glycol; and after dialysis to remove chemicals, the samples are lyophilized.

In addition, alternate methods of isolation and purification are being studied.

Three different assay systems are used to measure inhibitor activity--(1) effect of inhibitor on sarcoma and leukemia virus RNA-dependent DNA-polymerase activity in vitro; (2) effect of inhibitor on virus production using the XC plaque assay in vitro; and (3) effect of inhibitor in vivo on MSV or FLV induced tumors or leukemias.

Major Findings:

RLV infected cell ($JLSV_5$, $JLSV_{10}$) culture supernatants produced statistically greater inhibitor of viral replication (measured by XC plaque assay) than did controls ($JLSV_6$, $JLSV_9$). However, controls alone also produced some inhibition. In animals, some slowdown in the onset of RL(MSV) induced tumors was noted but this effect was of short duration. No preparation produced syncytia in the absence of added MLV or tumors in the absence of inoculation with RL(MSV).

Significance to Biomedical Research and to the Program of the Institute:

Isolation of natural products capable of inhibiting viral induced carcinogenesis is of importance as a tool for understanding the molecular basis of carcinogenesis and could ultimately prove of value in restricting viral-induced carcinogenesis.

Proposed Course:

Inhibitor preparations are being prepared from other cell lines for study and will be tested in each of the three assay systems described above. In addition, extracts of cells are also being tested for activity.

Honors and Awards:

Appointed to Editorial Board of Mechanisms of Aging and Development, Elsevier Press.

Publications:

Portugal, F.H.: Elution profiles of lysine and tyrosine transfer RNA during development. Mech. of Aging and Development, in press.

Portugal, F.H.: Specificity of lysine and tyrosine transfer RNA fractions for RNA codons. Mech. of Aging and Development, in press.

Serial No. NCI 4927
1. Viral Carcinogenesis Branch, OASDVO, Etiology
2. Ecology & Epizoology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Quantitative Microscopic Survey and Ultrastructural Studies of Viruses in Various Human and Animal Tissues

Previous Serial Number: Same

Principal Investigator: Dr. Victor H. Zeve

Other Investigators: None

Cooperating Units: Dr. Adeline Hackett, University of California
Dr. Brian Henderson, University of Southern California
Dr. Murray Gardner, University of Southern California

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description

Objectives:

(1) Intensive search for type B and C RNA viruses in human fetal, cancer and normal tissues, as well as human milk.

(2) To develop and utilize quick techniques for screening human breast tissue and milk for the presence of B particles as part of a comprehensive survey to elucidate the role of the B particle in human cancer.

(3) To clarify and define the ultrastructure of type A, B, and C RNA viruses and syncytial viruses positively for identification purposes.

Methods Employed:

New viral isolates and human milk specimens are being subjected to several fixation techniques for EM to determine if virus fixation techniques can alter ultrastructural findings, and to develop the most efficacious rapid scanning techniques. This work is being done in cooperation with

Dr. Robley Williams (NCI contract 71-2173) and the Jet Propulsion Laboratory at the California Institute of Technology. Well characterized RNA viruses are run in parallel as contrbls. Selected representative viruses in thin section are subjected to reduced electron bean intensity and accelerating voltages to determine the extent of beam damage to the specimen. Image intensification and enhancement by electron storage is being utilized to record images on photographic plates.

Major Findings:

Ultrastructural changes are observed as a result of varying fixation techniques, i.e., over-fixation in chrome osmium alters the appearance of the outer coat of simian foamy virus to the extent that the spiked structure is obliterated. Frozen milk specimens have given uniformly negative results, and experiments to fix and study fresh milk are underway.

Studies in collaboration with Dr. Hackett (NCI contract 63-13) on a "nonproducer" BALB/3T3 cell line transformed by murine leukemia virus have revealed unique cellular changes, but no evidence of virus. The negative results in EM are consistent with negative results in a number of other standard laboratory tests for virus in this cell line. Additional EM studies will be done at intervals.

Human cancer and fetal tissues have remained uniformly negative, and this work will not be continued on a routine basis, but rather will be confined to tissues of specific interest. This does not apply to the breast cancer program where evidence of B particles has been reported.

Significance to Biomedical Research and the Program of the Institute:

The association of a type B RNA virus with human breast cancer must be clarified. If the particle is indeed etiologically associated with cancer, it is important to develop methods for rapidly screening human materials to identify "carriers," and promote early diagnosis in cancer-prone individuals. This would also have important implications for designing approaches to breast cancer prevention and control. Since breast cancer is the leading cause of cancer deaths in women, with the incidence rising, these studies are of immediate relevance. Should the association between the B particle and cancer prove to be an artifact, this would provide reassurance to women who tested "positive" and would redirect research efforts.

Proposed Course:

Major emphasis will focus on the breast cancer screening program. Studies will be performed at the University of Southern California (NCI-68-1030) to take advantage of the excellent facilities and availability of human materials.

Honors and Awards: None.

Serial No. NCI-4928
1. Viral Carcinogenesis Branch, OASDVO, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: (A) Studies with Syncytium-forming Viruses
(B) Immunologic Studies of Virion Antigens

Previous Serial Number: SAME

Principal Investigator: Dr. Wade P. Parks

Other Investigators: None

Cooperating Units: Inside NIH:

Dr. Stuart A. Aaronson, NCI
Dr. Robert J. Huebner, NCI
Dr. Edward M. Scolnick, NCI
Dr. George J. Todaro, NCI

Outside NIH:

None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

(A) Further characterization of syncytium-forming viruses and their comparison with other mammalian viruses which have a reverse transcriptase.

(B) Preparation and characterization of immunological reagents which are specific for viral proteins and the development of techniques which improve the sensitivity and specificity of immunological assays.

Methods Employed:

Standard tissue culture, virologic, biochemical, and immunological procedures were employed where available.

Major Findings:

(A) The syncytium-forming viruses have several biophysical biological properties which suggest they may be related to other mammalian viruses with a reverse transcriptase. Employing the plaque assay technique which we developed, it has been possible to quantitate the effect of severe chemical and physical agents on these ubiquitous RNA-containing viruses. The viruses are very heat labile with a half-life at 37°C of 0.5 hours. On the other hand, the syncytium-forming viruses are extremely resistant to ultraviolet light inactivation (D_{37} = 2100 ergs), a feature previously reported for Type C viruses of avian and mammalian classes as well as visna virus. To date only viruses with a reverse transcriptase show this resistance suggesting a possible in vitro repair function for the reverse transcriptase. X-irradiation inactivation of the primate foamy virus type 3 suggested an RNA genome molecular weight of 1-2 x 10^6 daltons significantly smaller than the 3-4 x 10^6 dalton determination for both the murine leukemia and sarcoma viral genomes. These determinations contrast with the physical measurements of 1 x 10^7 daltons as measured by sedimentation velocity and suggest that both Type C viruses and the syncytium-forming viruses may have a segmented genome.

Studies with BrdU and IdU, analogues of the DNA base thymidine, revealed that 3 µg/ml of BrdU was an effective inhibitor of infectious virus (>3 log reduction in 72 hour yields). IdU was much less effective. Since BrdU is structurally very much closer to thymidine than IdU, the foamy virus polymerase may be able to discriminate between the two analogues. These data taken with the previously reported sensitivity to actinomycin D and the presence of RNA in the virion strongly suggest that replication of viruses with a reverse transcriptase employs a DNA intermediate and that all viruses with the reverse transcriptase share the biological consequences of reverse transcription.

A unique property of the foamy viruses from all species examined is the formation of intranuclear antigen in the nucleus of cells infected with these viruses. We have examined cells infected with feline, bovine, and primate viruses, and they all have nuclear antigen detectable by immunofluorescence. These data further document the existence of a nuclear phase for these unique viruses.

(B) Antibody to purified internal antigens of the Type C viruses has been prepared for two purposes. First, it is increasingly evident that immunological studies are essential to the classification of unknown viruses; and secondly, immunological methodology may be employed as a probe to detect viral specific proteins in human cells. We originally described antibody to partially purified RNA-instructed DNA polymerase (reverse transcriptase) of murine leukemia viruses. It was of interest that the antibody also partially inhibited the polymerase activity of viruses from rat, hamster, and feline Type C viruses. We have subsequently demonstrated that antibody prepared against the reverse transcriptase of avian Type C viruses and feline Type C viruses also have interesting patterns of inhibition. All avian Type C viral reverse transcriptases are inhibited by antibody to the avian enzyme but mammalian and reptilian viral enzymes are not. Conversely, the anti-polymerase sera against murine leukemia and feline leukemia viruses inhibits only the mammalian Type C viruses and does not affect the reverse transcriptase of avian or reptilian Type C viruses. These studies indicated that the reverse transcriptase had a broad interspecies reactivity and might be useful in classifying other viruses with reverse transcriptases as viral. For example, R-35 virus, purported to be a rat mammary tumor agent, was shown to be a Type C virus by this assay. However, the murine mammary tumor virus, visna virus, foamy viruses, and the Mason-Pfizer monkey virus (MP-MV) contained enzymes which appeared immunologically distinct. The subsequent discovery of the primate Type C viruses whose enzymes were also not inhibited by available antisera suggest that the failure might have been due to immunological diversity for the MP-MV, foamy, and visna viruses.

Employing these reagents we have been able to show that one putative human Type C virus had a murine enzyme and that another was not inhibited by available antisera. Another candidate human Type C virus was found to be a Chinese hamster virus by its patterns of inhibition. Yet, another human cell culture was discovered to have the DNA polymerases of EBV and mycoplasma in the supernatant fluids.

The second internal protein selected for study was the gs protein or viral polypeptide 3 (VP3) based on its migration in polyacrylamide gel electrophoresis. The VP3 has been reported to contain both interspecies (species specific) reactivities and interspecies reactivities common to all mammalian Type C viruses. Available techniques lacked adequate sensitivity and specificity to precisely define cross-reactions; therefore, we have investigated radioimmunoassay techniques

for the detection of the VP3 in virions and in cells. First, it was necessary to purify the antigen and prepare antisera against highly purified VP3. These reagents were used to prepare antisera to murine and feline VP3. The usefulness of such reagents was shown when used to identify ESP-1 as mouse and the primate Type C viruses as distinct from murine and feline viruses. The radioimmunoassay precipitation assay (RIPA) which is 1,000 times more sensitive than complement fixation and 40,000 times more sensitive than immunodiffusion for measuring viral protein is so specific that any sera with reactivity against the VP3 can be employed regardless of the other reactivities which are present. Employing RIPA, we can detect murine VP3 antigen in a wide variety of murine tissues and tissue culture cells. We have intensively studied the species specific and interspecies reactivity and conclude that they must reside on a single polypeptide. We have therefore developed a RIPA which measures only the interspecies reactivity. With this assay all the mammalian Type C viruses can be recognized and consequently identified. In agreement with the immunological studies with the reverse transcriptases, visna, murine mammary tumor virus, MP-MV, and the foamy viruses do not possess the mammalian interspecies Type C reactivity.

Significance to Biomedical Research and to the Program of the Institute:

(A) Latency, wide-spread prevalence, persistence and vertical transmission characterize natural infections with the syncytium-forming viruses. In view of their morphological, biological, and biochemical similarities with the more widely recognized "tumor" viruses with a reverse transcriptase, it seems probable that these viruses have a role in diseases of primates. The studies from this laboratory have contributed to the understanding that these agents are highly worthy of further study as possible disease producing viruses.

(B) The ability to identify unknown viruses and to detect cross-reacting viral antigens in natural tissues is the cornerstone of animal virology. The work presented here represents one of the few ongoing efforts to bring quantitative measurements to the immunology and classification of RNA-containing viruses which may be important in oncogenesis. The NCI programs generate many viral isolates. Experience, plus the number and variety of reagents and tests available almost nowhere else, makes possible their rapid identification.

Proposed Course:

(A) Attempted isolation and natural history studies with human syncytium-forming viruses.

(B) 1. Preparation of reagents to the reverse transcriptase of human candidate Type C viruses and the primate Type C viruses.

2. Application of the radioimmune precipitation assay to the detection and measurement of viral antigens in tissues from mammalian species.

(C) Preparation of reagents to the murine mammary tumor virus.

(D) Studies on transforming proteins.

Honors and Awards:

None

Publications:

Gilden, R. V., Parks, W. P., Huebner, R. H., and Todaro, G. J.: Murine leukemia virus group specific antigen in the Type C viruses-containing human cell line, ESP-1. Nature 233: 102-103, 1971.

Parks, W. P., Scolnick, E. M., Ross, J., Todaro, G. J., and Aaronson, S. A.: Immunologic relationships of reverse transcriptase from ribonucleic acid tumor viruses. J. of Virol. 9: 110-115, 1972.

Scolnick, E. M., Parks, W. P., Todaro, G. J., and Aaronson, S. Immunological characterization of primate Type C virus reverse transcriptases. Nature New Biology 235: 35-40, 1972.

Livingston, D. M., Scolnick, E. M., Parks, W. P., and Todaro, G. J.: Affinity chromatography of RNA tumor virus reverse transcriptase on a solid phase immunoadsorbent. Proc. Nat. Acad. Sci. U.S.A. (In Press).

Serial No. NCI-4929
1. Viral Carcinogenesis Branch, OASDVO, Etiology
2. Solid Tumor-Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Herpesviruses: Immunologic and Biologic Studies in Relation to Human Tumors

Previous Serial Number: SAME

Principal Investigator: Dr. Berge Hampar

Other Investigators: None

Cooperating Units: Inside NIH:

None

Outside NIH:

Dr. L. M. Martos, Flow Laboratories, Inc.
Dr. J. G. Derge, Flow Laboratories, Inc.
Dr. R. V. Gilden, Flow Laboratories, Inc.
Dr. K. C. Hsu, Columbia University
Dr. A. Girardi, Wistar Institute

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

(1) To study the mechanism of herpesvirus latency in human cells, with emphasis on the Epstein-Barr virus associated with Burkitt's lymphoma.

(2) To determine the prevalence of the herpesvirus genome in malignant and non-malignant cells by utilizing activation procedures developed in this laboratory.

(3) To study the relationship between herpesviruses and RNA-containing Type C viruses in human tumors.

(4) To characterize the antigens associated with herpesviruses and Type C viruses by immunoferritin and immunoperoxidase.

Methods Employed:

Standard tissue culture and virologic methods were employed.

Major Findings:

(1) Persistence in human cells of a repressed Epstein-Barr viral genome and activation by treatment with 5-bromodeoxyuridine:

Studies were undertaken to obtain evidence for a persistent Epstein-Barr virus (EB virus) genome in human cells and to develop methods for activating the repressed genome. The first study utilized the EB virus-positive P3HR-1 cell lines. The cells were made resistant to 100 µg/ml of 5-bromodeoxyuridine (BrdU) over a 6 month period. The drug resistant P3HR-1(BU) cells showed thymidine kinase activity at levels of 5-10% of the levels found in control P3HR-1 cells. Similarly, P3HR-1(BU) cells incorporated ^{3}H-thymidine and ^{3}H-BrdU at 1-2% of the levels seen with P3HR-1 cells. P3HR-1(BU) cells maintained on drug concentrations of 30 µg/ml and higher showed EB virus-associated antigen synthesis but no virus particles. Virus particles did reappear within 4 days after removal of the drug. Immunofluorescent and autoradiographic studies indicated that thymidine kinase activity was limited to those cells actively producing virus-associated antigen. The DNA produced in P3HR-1(BU) cells was labeled simultaneously with ^{14}C-thymidine and ^{3}H-thymidine to measure DNA synthesis by both thymidine kinase positive and negative pathways. Isopycnic banding in CsCl showed two radioactive DNA peaks. The first, and major peak, contained most of the incorporated ^{14}C-adenine and banded at a density of 1.69 g/cm^{3}, corresponding to the density of human DNA. The second, and minor peak, contained most of the incorporated ^{3}H-thymidine and banded at a density of 1.71 g/cm^{3}, corresponding to the density of EB virus DNA. Kinetic studies indicate that activation of thymidine kinase is followed first by synthesis of cell DNA followed by synthesis of "viral" DNA with concomitant switch-off of cell DNA synthesis. These studies indicate that (a) a repressed EB viral genome persists in P3HR-1(BU) cells that do not show thymidine kinase activity, (b) activation of the EB viral genome is accompanied by virus replication and the appearance

of thymidine kinase, and (c) thymidine kinase activity in P3HR-1(BU) cells can serve as a marker for EB viral genome expression.

The second study utilized the EB "virus-negative" Raji cell line. The Raji cells were made resistant to 100 µg/ml of BrdU over a 6 month period. Following removal of the drug, thymidine kinase activity was detected by autoradiography in 1-2% of the cells in the absence of virus antigen synthesis. Within several weeks after removal of the drug, however, EB virus associated antigens were detected by immunofluorescence and this was followed by the appearance of virus particles by electron microscopy. Subsequent to the appearance of EB virus antigens, thymidine kinase activity in Raji(BU) cells was limited to antigen producing cells. The DNA in Raji(BU) cells simultaneously labeled with ^{3}H-thymidine and ^{14}C-adenine showed one radioactive peak by isopycnic banding prior to the appearance of virus antigen. Subsequent to EB virus antigen synthesism, two radioactive DNA peaks were evident. The first, and major peak, contained the majority of the incorporated ^{14}C-adenine and banded at a density corresponding to that of human DNA, while the second, and minor peak, which contained the majority of the incorporated ^{3}H-thymidine banded at a density corresponding to that of EB virus DNA. These studies indicate that (a) "virus-negative" Raji(BU) cells carry a repressed EB viral genome which can be activated by treatment of the cells with BrdU, and (b) at least some Raji(BU) cells must contain a complete EB viral genome, since virus particles are synthesized in activated cells.

The sequence of EB virus antigen synthesis in Raji(BU) cells was studied by immunofluorescence using human sera containing antibodies to the "early" antigen complex and virus-associated antigens. Activation of the EB viral genome was characterized by the appearance of "early" antigens, while virus-associated antigens did not appear until virus particles were apparent by electron microscopy.

Clonal isolates of Raji(BU) cells have also been tested for the presence of the EB viral genome. These studies are still underway; however, of 20 isolates tested thus far, 14 have already shown evidence of "early" antigen synthesis. Assuming that all clonal isolates will eventually show evidence of EB virus, we may conclude that most, if not all Raji cells contain a complete EB viral genome.

Studies have been initiated to determine the optimal conditions for EB virus activation by BrdU. The initial

studies described above utilized cells which had been treated for long periods with increasing drug concentrations. Preliminary findings indicate that activation can be effectively accomplished without making the cells resistant to BrdU. Four "virus-negative" cell lines have been tested to date, and in each instance treatment for 7 days with drug concentrations of 2-10 μg/ml has resulted in activation of "early" antigen synthesis. Continued treatment with low concentrations of drug results in virus-associated antigen synthesis and the appearance of virus particles. These findings suggest that the EB viral genome can be activated by relatively short-term treatment with low doses of BrdU.

One of the EB "virus-negative" cell lines in which virus activation has been accomplished was derived from the pleural effusion of a woman with breast cancer. This cell line may also contain a Type C virus, suggesting that the same cell may contain a repressed RNA Type C viral genome and a DNA EB viral genome.

(2) Use of the immunoperoxidase technique for detecting herpesvirus antigens and antigens associated with Type C viruses:

The immunoperoxidase technique by light microscopy has been successfully adapted for identification of antigens associated with herpes simplex virus, Varicella-Zoster virus, human and simian cytomegaloviruses, and EB virus. In each instance the results by immunoperoxidase were comparable or better than results by immunofluorescence. The immunoperoxidase technique was also adapted for identification of group specific (gs 1) and interspecies specific (gs 3) antigens of RNA tumor viruses of murine, hamster and feline origin. Studies presently underway in collaboration with Dr. Girardi are testing the usefulness of the immunoperoxidase technique for studying human Type C viruses.

(3) Immunoferritin studies with Type C viruses:

Antisera specific for murine Type C virus envelope and gs antigens have been conjugated with ferritin and tested for reactivity with virus shedding cultures. The envelope specific antisera react with enveloped particles, but the gs specific antisera show no reactivity. Procedures are being tested for dissociating the viral envelope for determining the location of the gs antigen complex.

Significance to Biomedical Research and the Program of the Institute:

The successful activation of the EB viral genome opens the way for studies to determine the presence of this virus in a repressed state in human tumor cells. In addition, the way is now open for studying the effects of dual infection with herpesvirus and Type C virus, especially in cells where both virus genomes may be present in a repressed state.

Proposed Course:

Studies will continue to delineate the mechanism of herpesvirus integration and activation by BrdU.

Honors and Awards:

None

Publications:

Hampar, B., Derge, J. G., Martos, L. M., and Walker, J. L.: Synthesis of Epstein-Barr virus after activation of the viral genome in a "virus-negative" human lymphoblastoid cell (Raji) made resistant to 5-bromodeoxyuridine. Proc. Nat. Acad. Sci. (U.S.A.) 69: 78-82, 1972.

Hampar, B., Derge, J. G., Martos, L. M., and Walker, J. L.: Persistence of a repressed Epstein-Barr virus genome in Burkitt lymphoma cells made resistant to 5-bromodeoxyuridine. Proc. Nat. Acad. Sci. (U.S.A.) 68: 3185-3189, 1971.

Hampar, B., Gilden, R. V., Kelloff, G., Oroszlan, S., and Simms, D.: Immunofluorescent detection of murine and hamster Type C virus species-specific (gs-1) determinants by monospecific guinea-pig sera and interspecies-specific (gs-3) determinants by tumor-bearing rat sera. Int. J. Cancer 8: 425-431, 1971.

Hampar, B., Martos, L. M., and Walker, J. L.: Epstein-Barr virus in human lymphoblastoid cells: Enhancing the percentage of virus-positive cells by cocultivation with African green monkey (vero) cells. J. Nat. Cancer Inst. 47: 535-537, 1971.

Hampar, B., Hsu, K. C., Martos, L. M., and Walker, J. L.: Serologic evidence that a herpes-type virus is the etiologic agent of heterophile-positive infectious mononucleosis. Proc. Nat. Acad. Sci. (U.S.A.) 68: 1407-1411, 1971.

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Miyamoto, K., Morgan, C., Hsu, K. C., and Hampar, B.: Differentiation by immunoferritin of herpes simplex virion antigens with the use of rabbit 7S and 19S antibodies from early (7-day) and late (7-week) immune sera. J. Nat. Cancer Inst. 46: 629-646, 1971.

Serial No. NCI 4930
1. Viral Carcinogenesis Branch
OASDVO, Etiology
2. Field Studies Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Biology of C-type RNA Viruses in Naturally Occurring Neoplasias

Previous Serial Number: Same

Principal Investigator: Dr. Paul Arnstein

Other Investigators: Dr. Robert J. Huebner, VCB, NCI
Dr. Padman S. Sarma, VCB, NCI
Mr. H.C. Turner, VCB, NCI
Mr. John Estes, VCB, NCI

Cooperating Units: Outside NIH:
Dr. M. Gardner, USC School of Medicine, Cancer Research Laboratory, Los Angeles, California
Dr. R. McAllister, Children's Hospital, Los Angeles, California
Dr. E.H. Lennette, California State Department of Public Health, Berkeley, California

Man Years:

Total:	2.5
Professional:	1.0
Other:	1.5

Project Description

Objectives:

To determine prevalence and biological characteristics of C-type agents in human and animal neoplasia collected through NIH-NCI contracts in California and elsewhere: to develop in vitro and in vivo procedures for the detection of type C virus, viral antigens and viral genomes particularly as they relate to tumorigenicity in immuno-suppressed mice: to demonstrate production of antiviral antibody in animals with experimental ONCORNA virus-induced tumors or hyperimmunized with known viral preparations; develop tests to determine presence or absence of neutralizing antibody against C-virus in animals and humans with specified neoplastic diseases occurring spontaneously in California.

Methods Employed and Major Findings:

Feline sarcoma virus (FSV) replication and assay. Two strains of FSV, the ST strain (Snyder, Theilen, Nature 221: 1074, 1969) and the GA strain (Gardner, Arnstein et al, Nature 226: 807, 1970), have been adapted to growth in normal beagle embryo (NBE3) cells in which they produce easily recognizable foci of transformation. Stocks of these viruses have been produced which could be assayed accurately and were found to contain "transforming" and "helper" type virus components, as previously reported by Sarma for cat cell propagated FSV. In both the ST and the GA strains, the "helper" virus was in 100-1000-fold over the transforming virus, and could be produced free of the transforming virus by end point dilution. In collaboration with Dr. Riggs, the helper virus was repeatedly assayed by the indirect fluorescent antibody (IFA) test for envelope antigen of the feline type C virus. The titers were reproducible and demonstrated that the excess helper virus (beyond the focus-forming end point) remains at a constant level for both strains of FSV. Passage through cat, dog or primate cells did not result in production of FSV with a lower "helper sarcoma" ratio.

FSV neutralization experiments. Sera derived from animals hyperimmunized with feline ONCORNA virus preparations or bearing FSV tumors have been tested for focus neutralization against tissue culture grown FSV from NBE3 cells. Animals which had received preparations containing large amounts of viable GA FSV, such as tumor homogenates, viral concentrates prepared according to Moloney's method, or large doses of tissue culture grown virus were usually found to produce high levels of neutralizing antibody; up to 3 logs of GA virus, both "sarcoma" and "helper" (proven by focus reduction and IFA antigen titration) have been neutralized with select sera. Preliminary experiments suggest the neutralizing antibody to be relatively short-lived; representative sera from animals bearing subsequently regressive FSV sarcomas had detectable focus-neutralizing antibody at the peak of tumor growth, but were completely negative 3-4 months later when the tumors had disappeared.

Sera from animals with spontaneous neoplasms and other diseases, some of which had been confirmed positive for C-type particles, failed to neutralize significant multiplicities of FSV. These animals were submitted by cooperating veterinarians in the Los Angeles and Berkeley areas.

In vivo tumorigenesis tests. I. Antithymocyte serum (ATS)-treated mice. Immunosuppression of NIH Swiss mice by administration of ATS had made it possible to replicate certain transformed and tumor-derived cells, as well as some original tumor tissue, from heterologous hosts. The technique is based in Eric Stanbridge's article (Nature 221: 80, 1969) demonstrating the growth of HeLa cells in mice. Beginning with this known tumorigenic cell line, we have applied the technique to other cell and tumor types and added the following refinements: (a) all attempts at tumor cell growth are subjected to pathological examination by our veterinary pathologist; (b) all tumor-suspect lesions in ATS-treated mice are removed, grown up in

culture and submitted for karyology (with the aid of Dr. W. Nelson-Rees, NBL); (c) all successful tumors induced and removed are examined here, as well as submitted to appropriate cooperating units for detection of viral activation (either complete virus or viral products such as gs antigens, RNA-directed polymerase enzyme, envelope antigens).

The following observations have been made in pilot experiments: (1) "Normal" cells produced no tumors in either untreated or ATS-treated mice. Included were human diploid cells, normal fetal dog (BNE3) and cat cells and normal baboon cells. High and low passage levels were included in the series. (2) HeLa cells produced rapidly growing invasive carcinomas in ATS-treated mice, transient inflammatory response in untreated mice. (3) NBE3 (dog) cells transformed by FSV of both strains available (ST and GA) produced invasive fibrosarcomas in ATS-treated mice; cells grown from the tumors were identifiable as canine karyotype and still were producing FSV. No tumors were obtained when untreated mice received the same number of transformed cells. Similar fibrosarcomas were produced by inoculation of (4) whole minced tumors of puppies infected at birth with FSV, (5) baboon cells transformed by FSV, (6) whole minced feline fibrosarcomas, (7) NBE3 cells infected with nontransforming "helper" feline C-type virus produce no tumors in ATS mice, (8) two of four human colon carcinomas produced subcutaneous mucus-producing carcinomas in 3 of 12 ATS mice, the tumors were not progressive and the human cells could not have been grown out from them. (Future tests on carcinomas will utilize younger mouse litters). (9) Human rhabdomyosarcoma (RD) cells produced sarcomas intracerebrally, subcutaneously and intraperitoneally in ATS mice. Untreated mice given RD cells did not grow tumors. Cells grown out of all tumors had the human karyotype, typical of the RD. Viral and antigenic tests on these are in progress. (10) Three additional "fresh" human tumors (2 carcinomas and 1 fibrosarcoma) and 10 tissue cultures derived from a variety of human tumors, in low passage levels (3-8) failed to produce tumors in ATS-treated mice. (The tumors may have been held too long, and the tissue culture process may have favored stromal versus the neoplastic cells).

II. Other in vivo results. Serially passaged transformed NBE3 cells (producing GA FSV) have been inoculated into puppies and have proven fibrosarcoma inducing. In cooperation with Dr. J. Melnick at Baylor University, baboon testis cells have been obtained (orchidectomy), transformed *in vitro* by GA FSV and ST FSV and returned for inoculation into the donor baboons. Both inoculated baboons showed antibody response (CF tests versus feline ONCORNA virus antigen), but no tumors were produced.

Significance to Biomedical Research and to the Program of the Institute:

An improved neutralization for FSV, particularly if rendered more sensitive, may offer a survey tool which would later be applicable to other animal species, perhaps even as a tool for testing for antibody among known cancer patients, against human candidate ONCORNA virus isolates or their pseudotypes.

The immunosuppressed mouse (ATS-treated) used to identify and reject stromal cells from cell cultures and fresh tumors, and permit good outgrowth of neoplastic cells promises to be a tool in narrowing down the expensive and time-consuming efforts of seeking human oncogenes, tumor virus products, or virions to cells which are most likely to yield such agents; mouse passage may even serve to activate the virus from the "switched off" tumor cell, as demonstrated by McAllister and co-workers for the RD cells through feline passage.

Tumors capable of reproducing the same tumor type in the ATS mouse should be especially "targeted" for additional attempts at virus detection and/or activation. The technique may also prove adaptable to testing selected anti-cancer drugs on known human tumors which can be observed actively growing on a well-defined, non-human host.

Proposed Course:

Continue and amplify above studies, particularly: (a) improve sensitivity of the neutralization test for FSV; (b) apply technique to "rescue" of human genomes; (c) further clarify tumorigenicity of various neoplasms, particularly human cancers, for ATS-treated mice and seek activation of human ONCORNA virus by these experiments; (d) test for "rescue" of human ONCORNA virus by furnishing murine C-virus templates (e.g., use viremic AKR mice instead of "switched off" NIH Swiss).

Honors and Awards:

None

Publications:

Gardner, M.B., Charman, H.P., Johnson, E., Rongey, R.W., Gilden, R., Arnstein, P., and Huebner, R.J.: Natural history studies of the feline tumor virus genome: Detection of group-specific antigen and C-type particles. Proc. 12th National Congress of Biologics Standards, Karger, Basel, in press.

Serial No. NCI 4931

1. Viral Carcinogenesis Branch
   OASDVO, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: (A) C-type RNA Virus Production in Somatic Cell Hybrids.
(B) Analysis of Proteins with Affinity for DNA in C-type Virus Infected Cells.

Previous Serial Number: None

Principal Investigator: Dr. Kenneth H. Rand

Other Investigators: Dr. Cedric Long, Flow Laboratories, Inc., Rockville, Maryland

Cooperating Units: Inside NIH:

Dr. Robert J. Huebner, VCB, NCI

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description

Objectives:

(A) Somatic cell hybrids are known to undergo loss of chromosomes as their passage in culture increases. Since there is good theoretical reason to believe that type C RNA viruses are integrated into the mammalian chromosome(s), carefully selected hybrid mammalian cells are being investigated for their ability to continue type C virus production in serial passage.

(B) Proteins with affinity for DNA have been analyzed and described in T4 infected E. coli, and certain mammalian cell lines during different phases of the growth cycle. It is the purpose of this project to apply these techniques to type C virus infected cells.

Methods Employed:

(A) The hybrid cell lines are produced as follows. One cell line is made resistant to BrdU. Enzyme assay shows these cells have lost thymidine kinase and hence cannot use BrdU, or thymidine. The other cell line is made resistant to 6-thioguanine and lacks the enzyme hypoxanthine-guanosine phosphoribosyl transferase (HGPRT). It is unable to utilize hypoxanthine. Cells are fused with inactivated Sendai virus. In selective media (HAT) containing hypoxanthine, aminopterin (to block de novo synthesis) and thymidine, both parental cell types are killed and only fused cells survive.

(B) Standard cell culture and virological techniques were used: DNA cellulose chromatography as described by Alberts; and SDS acrylamide gel electrophoresis.

Major Findings:

(A) Eight hybrid clones (THR clones) (see Long et al, 1972 for complete description) were established between HT-1 (carrying M-MSV) and TG-8(RLV) (a mouse cell line derived from 3T6, resistant to thioguanine and shedding a high titer of Rauscher leukemia virus). In similar fashion, four hybrid clones were isolated from the fusion of HT-1 and "Klein" (a cell line from a patient with Lesch-Nyhan syndrome, deficient in HGPRT [KLHT clones]). Using a slight modification, seven hybrid clones were made between HT-1 and human peripheral white blood cells from seven patients with leukemia (obtained from Dr. Robert McAllister, Children's Hospital, Los Angeles).

The THR clones have been most extensively investigated thus far. Of the eight, all possess enzymatic evidence of both hamster and mouse genetic material. In spite of this, one clone was found to produce no MSV, nor could it be rescued by direct infection with RLV. Leukemia virus production was high in this clone. Two other clones produced a 100-fold excess of sarcoma virus over leukemia virus. Subclones have been produced from one of these and are currently under intensive investigation. Recent results suggest that some have lost the ability to produce RLV entirely. Since chromosomes are shed as serial passage goes on, loss of leukemia virus expression could be due either to loss of the virus itself or to loss of a necessary gene function. The distinction is of great importance in view of the oncogene theory. We feel these possibilities can be directly tested by re-fusing the hybrid cell which has lost RLV with the parent line TG-8, without RLV, containing all of the normal host gene functions. If no virus appears, this will provide evidence that the viral genome itself has been lost as the chromosomes have been shed. Furthermore, if the hybrid can support RLV growth following re-infection, adequate host genes must be present.

The leukemic-HT-1 and "Klein"-HT-1 hybrids are currently being studied to determine whether the M-MSV genome is present, by means of rescue with RLV. It is conceivable that the leukemic-HT-1 hybrids might contain a "human"

leukemia virus capable of rescuing the M-MSV. So far, assay for focus-forming virus with human fibroblast tropism has been negative.

(B) Many technical problems have hindered immediate application of these methods. For instance, it is important to have highly radioactive, DNA-free, protein extracts. Consequently, large numbers of cells must be used, and special media lacking the amino acids to be incorporated into protein are also necessary. Furthermore, because of the many steps involved, an experiment can take as long as several weeks. At present, we have succeeded in producing the requisite highly radioactive protein extracts, and have obtained crude fractions with appropriate DNA binding ability.

Significance to Biomedical Research and to the Program of the Institute:

(A) Somatic cell hybrid production provides the opportunity to study post chromosomes-virus relationship in a manner previously impossible.

(B) Separation of DNA binding proteins by DNA cellulose chromatography should provide an excellent means of isolating new proteins essential to type C virus expression.

Proposed Course:

(A) (1). We expect to do chromosome analysis on the hybrid clones to determine the relative preponderance of mouse and hamster chromosomes. In addition, we plan to make hybrids between a rat kangaroo cell line and a mouse line. Since the chromosomes are so vastly different morphologically, localization of the viral genome to a specific chromosome may be possible. (2). In studying the human-HT-1 hybrids, use of BrdU to induce a "helper" virus from the human half of the hybrid may lead to rescue of the M-MSV with human tropism. This possibility will be investigated for the "Klein"-HT-1 and the leukemic-HT-1 hybrids by testing for reverse transcriptase activity and focus-forming ability on human diploid fibroblasts.

(B) Studies will continue toward the objectives indicated above.

Honors and Awards:

None

Publications:

None

Serial No. NCI 4932
1. Viral Carcinogenesis Branch
OASDVO, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Alterations in tRNA Species During Oncogenic Viral Infection.

Previous Serial Number: None

Principal Investigator: Dr. Josephine Simonds

Other Investigators: Dr. Franklin Portugal
Dr. Bernice Moll

Cooperating Units: Inside NIH:

Dr. Robert J. Huebner, VCB, ET, NCI
Dr. Stuart Aaronson, VLLB, ET, NCI
Mr. William Lane, VCB, ET, NCI

Outside NIH:

Dr. G. Spahn, Microbiological Associates, Inc.

Man Years:

Total:	1.2
Professional:	1.0
Other:	0.2

Project Description

Objectives:

To determine whether alterations in isoaccepting species of tRNA occur in mammalian cells during infection with RNA (MSV or MSV/MLV) or DNA (SV40) containing viruses.

Methods Employed:

Transfer-RNA isolated from each infected cell line was acylated in vitro by standard methods and mixed with RNA from non-infected cells charged with the corresponding amino acid isotope. Double-label chromatography profiles were determined by precipitating acylated tRNA from each fraction and collecting on millipore filters.

Major Findings:

No clearly unique species of tRNA has been observed in any of three virus infected cell lines that is absent in control (non-infected) cells, after profiles for alinine, arginine, aspartic acid, histidine, isoleucine, leucine, lysine, phenylalanine, serine, tyrosine, and valine were examined. However, a number of significant differences were noted when non-infected cells from 14-17 day old embryos were compared to newborn BALB/c mice. This confirms earlier studies we have carried out on other species that indicate significant alterations occur during embryonic development. The results also suggest at least one fundamental difference between embryological development and viral carcinogenesis.

Significance to Biomedical Research and to the Program of the Institute:

The alterations, if any, of mammalian cell metabolism during oncogenic viral infection are poorly understood. In bacteria, alterations in certain iso-accepting species of tRNA during phage infection provides the virus with an important mechanism for regulating cell metabolism. The present study seeks to determine whether viruses infecting mammalian cells contain information to produce a similar regulatory effect. This information would be of value for understanding the mechanism of cell transformation and viral carcinogenesis.

Proposed Course:

Project is presently nearing completion and is being prepared for publication. Codon assays are being carried out to determine the significance of alterations which occur as the result of embryonic development in the mouse.

Honors and Awards:

None.

Publications:

None.

Serial No. NCI-4933
1. Viral Carcinogenesis Branch
OASDVO, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Alterations of Polysome Profiles During Transformation.

Previous Serial Number: None

Principal Investigator: Dr. Bernice Moll

Other Investigators: Dr. Franklin Portugal
Dr. Josephine Simonds

Cooperating Units: Inside NIH:

Dr. Robert J. Huebner, VCB, ET, NCI
Dr. Stuart Aaronson, VLLB, ET, NCI
Mr. William Lane, VCB, ET, NCI

Outside NIH:

Dr. G. Spahn, Microbiological Associates, Inc.
Electronucleonics, Inc.

Man Years:

Total:	0.9
Professional:	0.7
Other:	0.2

Project Description

Objectives:

The behavior of cells transformed by certain temperature sensitive mutants of avian sarcoma viruses suggest that in these cells transformed by an RNA tumor virus, a viral function is needed continuously for the transformed state to be maintained. It is proposed to attempt the identification of proteins which may have a similar function in mouse cells transformed by a murine sarcoma virus by comparing the proteins in transformed cell lines with those in a nontransformed cell line. If the total proteins of transformed cells were to be studied, the presence of those virus-specified proteins which induce and/or maintain the transformed state might very likely be obscured by a high background of numerous cell-coded proteins. Therefore, attempts will be made to isolate a specific class of polyribosomes associated with virus-specified nascent proteins. The identification of a class of

polyribosomes associated with virus-specified proteins will be facilitated by the use of antisera to structural proteins of the virion. In order to help distinguish proteins responsible for inducing and/or maintaining the transformed state from structural proteins of the virion, two types of virally transformed cell lines will be examined; one which produces virus particles, and one which fails to produce virus particles. Additional experiments will be performed to determine which proteins absent from the nontransformed mouse cell line and present in both virally transformed mouse cell lines can be detected in hamster and human cell lines which have been transformed by the same murine sarcoma virus.

Methods Employed:

(1) Examine polyribosomes of C, NPT, and PT cells in an attempt to detect by size difference a class of large polyribosomes (such as those found in Rauscher virus-infected mouse spleen cells) in NPT and PT cells which are absent from or present in reduced amounts in untransformed cells: (2) React polyribosome fractions from PT cells with antisera to murine group-specific antigens and to the viral envelope antigens in an attempt to identify a class of polyribosomes enriched for polyribosomes associated with viral rather than host cell nascent proteins: (3) Dissociate nascent proteins from the polyribosome class containing viral antigens and purify these proteins by polyacrylamide gel electrophoresis.

Major Findings:

No significant findings as project has just been initiated. A peak of large polysomes was observed in one of the transformed cell lines that was not detectable in the nontransformed cell line in several experiments. However, in several other experiments this peak was not observed. Efforts are now being directed toward analyzing and eliminating the factor(s) responsible for the variability in the observed polyribosome profiles from the same cell line.

Significance to Biomedical Research and to the Program of the Institute:

The isolation and characterization of proteins specified by the genome of RNA tumor viruses which are responsible for the induction and/or maintanance of the oncogenic state could prove useful in the early diagnosis of cancerous or precancerous states. The characterization of such proteins would also be useful for studies of the mechanism whereby the viral genome becomes expressed.

Proposed Course:

BALB/3T3 cells, and producer as well as nonproducer cells (prepared from the same cell clone), will be examined by density gradient centrifugation for polysome profiles. Gel electrophoresis will be used to separate protein components from selected regions of the polysomes.

Honors and Awards: None

Publications: None

Serial Number NCI/NIAID 71 D
1. Viral Diseases
2. Viral Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies of Mouse Leukemia Viruses

Previous Serial Number: Same

Principal Investigators: Dr. Janet W. Hartley, LVD, NIAID
Dr. Wallace P. Rowe, LVD, NIAID

Other Investigators: Dr. Natalie M. Teich, LVD, NIAID
Dr. Douglas R. Lowy, LVD, NIAID

Cooperating Units: Inside NIH:
Dr. Arthur S. Levine, MB, NCI

Outside NIH:
Dr. John C. Parker, Microbiological Associates, Inc., Bethesda, Md.
Dr. Edward A. Boyse, Sloan-Kettering Institute for Cancer Research, New York, N. Y.

Man Years:

Total:	11.1
Professional:	3.9
Other:	7.2

Project Description

Objectives:

To utilize sensitive virus isolation and quantitation procedures for murine leukemia and sarcoma viruses in studying the natural history of infection with these agents and the biology of their growth in tissue culture.

Methods Employed:

Tissue culture procedures, using plaque and focus assays and complement fixing antigen induction; complement fixation; fluorescent antibody techniques; tumor induction in mice, hamsters and rats.

Major Findings:

Activation of murine leukemia virus (MLV) in cell cultures: Further studies with MLV-negative cell lines (derived from small numbers of embryo cells from the high virus AKR mouse strain) indicated that all AKR embryo cells growing in cell culture contain the full viral genome in an unexpressed form. This may be concluded from the demonstration that three such cell lines and all of 18 clones or subclones obtained from two of the lines have the capacity to produce infectious virus either spontaneously or after various types of manipulation such as X-irradiation, SV40 transformation, or treatment with certain chemicals. Spontaneous activation rates were of the order of 1 in 10^8 or 10^9 cells, while X-irradiation increased the rate to 1 in 10^5 cells. In contrast, exposure of the cells to the thymidine analogs 5-bromodeoxyuridine (BUDR) and 5-iododeoxyuridine (IUDR) increased activation rates dramatically with at least 1 cell in 1000 in some lines producing virus six days after treatment with IUDR. Induction of MLV by BUDR and IUDR appears to require incorporation of the analogs into DNA, since addition of 5-fluorodeoxyuridine at the time of exposure to BUDR or IUDR as well as exposure of BUDR treated cells to visible light increased the proportion of activated cells. Thymidine treatment prevented induction. By use of quantitation procedures involving appropriate cell and IUDR titrations it has been possible to detect induction rates as high as one virus-producing cell in 200. Kinetic studies of IUDR activation indicate that some cells are producing virus within 48 hours after initial exposure to the drug, with the number increasing markedly by 72 to 96 hours. The extent of activation of viral gene expression is greater than is indicated by the number of virus producing cells, since 10 to 30 times as many cells are positive for immunofluorescent stainable antigen at three days after exposure to IUDR.

Primary AKR embryo cells are activated by IUDR with an efficiency approximating that of AKR cell lines, and embryo cells derived from crosses of AKR with BALB/c mice (a B-type mouse, whose cells are resistant to the N-tropic AKR virus) are activated almost as efficiently.

In tests for IUDR inducible immunofluorescent stainable antigen and infectious virus in low leukemia incidence mice we have found that continuous lines of embryo cells from BALB/c and DBA mice are induced to form antigen in a large number of cells, but with only minimal production of infectious virus. Neither antigen nor virus has been induced in primary or secondary embryo cell cultures from these strains; negative results have also been obtained with secondary and continuous NIH Swiss embryo lines and a continuous wild mouse embryo line.

Quantitation of virus in AKR mice: Using the XC plaque procedure, the titer and organ distribution of MLV was determined in AKR mice of various ages. Infectious virus first appeared shortly before or after birth, and was continuously present thereafter. Highest infectivity titers were found in uterus and bone, with spleen slightly lower; titers in normal thymus were low, increasing significantly with development of thymic lymphoma. The level of viremia decreased after the first month of life but increased sharply in lymphomatous mice.

Genetics of MLV infection: In an attempt to determine the form in which genetic information coding for infectious MLV is transmitted, the appearance of infectious virus has been traced in the progeny of crosses and backcrosses of AKR mice with various N- and B-type partners, with different H_2 alleles. The results of this and other studies support the concept of germ cell transmission of a genetic element coding for infectious virus. The data thus far indicate that the AKR mouse contributes two unlinked genes, either of which gives a positive phenotype.

MLV in C57 BL mice: From the B-type C57 BL mouse strain, 5 isolates of N-tropic virus have been recovered, one from a 12 mos. old animal and four from mice 27 mos. old. Only B-tropic virus had previously been found in this strain.

Serology of MLV isolates from wild mice: Preliminary results of tests on two MLV isolates obtained from wild mice indicate that these agents are serologically distinct from viruses of the FMR or Gross-AKR subgroups.

Significance to Biomedical Research and the Program of the Institute:

The finding that IUDR and BUDR are highly efficient inducers of MLV in AKR cell lines provides a powerful tool for studies of leukemia virus infections in mice and other species. In addition to testing for activation of complete infectious virus, induction of synthesis of various viral proteins in negative cell lines can be studied, and it may be possible to utilize the technique in tests for oncogene expression.

Genetic studies of MLV infection have provided strong evidence that information for infectious virus is transmitted chromasomally. Establishment of the location of viral genes should make it possible to determine the relation of the virus loci to other loci coding for MLV-related antigens, and to study the role of genetic factors in determining tumor development.

Proposed Course:

The IUDR activation system will be utilized to explore further the qualitative and quantitative differences between high and low leukemic strains of mice. Studies of the mechanism of the activation process will be continued, with particular interest lying in the effect of cell synchronization, dose response curves, and tracing of biochemical events during activation.

Attempts will be continued to enhance activation rates and to apply more efficient procedures to cell cultures of human origin.

Genetic studies will emphasize the establishment and characterization of single gene families for use in linkage studies of the viral genes and other leukemia-associated genes.

Publications:

Pincus, T., Hartley, J.W., and Rowe, W.P.: A major genetic locus affecting resistance to infection with murine leukemia viruses. I. Tissue culture studies of naturally-occurring viruses. J. Exp. Med. 133: 1219-1233, 1971.

Pincus, T., Rowe, W.P., and Lilly, F.: A major genetic locus affecting resistance to infection with murine leukemia viruses. II. Apparent identity to a major locus described for resistance to Friend murine leukemia virus. J. Exp. Med. 133: 1234-1241, 1971.

Hartley, J.W., Rowe, W.P., and Wilsnack, R.E.: Detection of murine leukemia virus antigens. Proceedings, Tenth International Cancer Congress, May 1970, Houston, Texas, in press.

Levy, J.A. and Rowe, W.P.: Lack of requirement of murine leukemia virus for early steps in infection of mouse embryo cells by murine sarcoma virus. Virology 45: 844-847, 1971.

Rowe, W.P.: The kinetics of rescue of the murine sarcoma virus genome from a non-producer line of transformed mouse cells. Virology 46: 369-374, 1971.

Lowy, D.R., Rowe, W.P., Teich, N., and Hartley, J.W.: Murine leukemia virus: high frequency activation in vitro by 5-iododeoxyuridine and 5-bromodeoxyuridine. Science 174: 155-156, 1971.

Rowe, W.P., Hartley, J.W., Lander, M.R., Pugh, W.E., and Teich, N.: Non-infectious AKR mouse embryo cell lines in which each cell has the capacity to be activated to produce infectious murine leukemia virus. Virology 46: 864-874, 1971.

Rowe, W.P., and Pincus, T.: Quantitative studies of naturally occurring murine leukemia virus infection of AKR mice. J. Exp. Med. 135: 429-436, 1972.

Rowe, W.P., Lowy, D.R., Teich, N., and Hartley, J.W.: Some implications of the activation of murine leukemia virus by halogenated pyrimidines. Proc. Nat. Acad. Sci., in press.

BIOHAZARDS CONTROL AND CONTAINMENT SEGMENT

Dr. Alfred Hellman, OASDVO, Etiology Area, Chairman

Mr. William Emmett Barkley, OASDVO, Etiology Area, Vice Chairman

THE DOW CHEMICAL COMPANY (PH 43-65-1045)

Title: Research and Development of Biohazards Containment Facilities

Contractor's Project Director: Mr. Cyril B. Henke

Project Officer (NCI): Mr. W. Emmett Barkley

Objectives:

1. In collaboration with the NCI Office of Biohazards and Environmental Control, the contractor performs Biological Safety and Environmental Control surveys of SVCP contractor operations to evaluate laboratory practices, safety equipment and facilities and to assess the effect of contractor operations on laboratory safety and environmental quality.

2. The contractor assists the Office of Biohazards and Environmental Control in the preparation and dissemmination of safety equipment specifications, facility design criteria, operations guidelines and safety procedures. The contractor also prepares engineering design drawings and specifications for containment systems required to meed specific program needs.

3. The contractor operates a safety equipment certification program to assure safe operation of SVCP safety and containment equipment.

4. The contractor assists the Office of Biohazards and Environmental Control in the review of plans and specifications for SVCP renovation and new facility construction projects.

Major Findings: The contractor participated in ten biological safety and environmental control surveys and has developed significant recommendations to improve the environmental quality of SVCP laboratory operations. Survey follow-up activities helped to eliminate potential safety and environmental control

problems experienced by a few of the contractors surveyed. The following examples highlight these activities: (1) containment systems were designed to eliminate potential hazards associated with oncogenic virus production, (2) recommendations for improved safety operation of the K-II centrifuge were developed, (3) recommendations for improving quality control practices of virus production activities were developed, (4) certification of in-use biological safety equipment was performed at the majority of survey sites.

The contractor assisted NCI in the development of minimum standards for biological safety and environmental control.

Significance to Biomedical Research and the Program of the Institute: This contract contributes biological safety and environmental control expertise to the SVCP. This expertise is used to improve the quality and safety of the cancer research laboratory environment. The contractor functions as an integral part of the NCI Office of Biohazards and Environmental Control.

Proposed Course: The contractor will continue to provide technical assistance to the SVCP contractors on problems of environmental control, personnel safety and product protection. The contractor will participate on approximately twenty-five Biohazard Safety and Environmental Control surveys. Plans for expanding the contractor's engineering design and review support activities will be initiated this year.

Date Contract Initiated: June 25, 1965

Current Annual Level: $200,000

OHIO STATE UNIVERSITY (PH43-65-1001)

Title: Biohazard Control and Containment in Oncogenic Virus Research

Contractor's Project Director: Dr. D. Yohn

Project Officers (NCI): Dr. A. Hellman
Dr. A. K. Fowler

Objectives: The purpose of this research program is to elucidate the possible hazards to man and animals from exposure to oncogenic viruses and their infectious nucleic acids. In addition the possibility that animal hosts are able to respond immunologically to their homologous oncornaviral antigens is being investigated and efficacy of various vaccine preparations is being tested.

Major Findings: All the virus parenteral and aerosol experiments have been completed. Three of eight groups of animals exposed to infectious nucleic acid remain in experiment. These include: 137 Swiss mice injected subcutaneously, 102 newborn and 102 thymectomized weanling mice exposed by the aerosol route, and 19 newborn and 82 weanling thymectomized PD_4 hamsters also exposed to an aerosol of infectious nucleic acid. All the remaining animals have been necropsied, gross lesions examined histologically, and terminal sera from the last groups are currently being tested by immunofluorescence for the presence of SV_{40} T-antibody. In the parenteral studies we have demonstrated that infectious SV_{40} DNa prepared from our semi-purified virus is oncogenic and that the subcutaneous route is more sensitive than the intracerebral route for challenge with either virus or nucleic acid. There were, however, fewer tumors in animals injected with infectious nucleic acid than those receiving whole virus. No tumors have appeared in the mice injected with SV40 infectious nucleic acid. No new tumors have developed in the aerosolized hamsters and mice.

In general, all the groups of hamsters and mice show evidence of infection by the presence of SV_{40} specific antibody. The control hamsters and mice are negative. All the tumor bearing animals in the parenterally injected groups as well as some non-tumor bearing animals have T-antibody.

Feline Leukemic Horizontal Transmission:

1. Intranasal transmission - The experiments demonstrating that feline leukemia is transmissible by a single intranasal inoculation of neonatal kittens are completed. One of 4 gnotobiotic kittens inoculated intranasally with FeLV in a final experiment to corroborate previous data has died of lymphosarcoma at 397 days of age. The remaining 3 cats are alive and free of clinical disease at 45 days of age.

Feline Virus Vaccine:

1. Immune reactivity of cats - This portion of the study, the definition of the immune response of the normal cat and cats with feline leukemia, was begun under the concept that the understanding of the pathogenetic mechanisms in feline leukemia, including response to an inactivated virus vaccine, would be aided by such knowledge. The objectives included identification, separation and purification of the immunoglobulin classes of the cat and quantitation of these classes in normal and leukemic cats of various ages. Also, humoral and cellular immune response would be evaluated following injection of sheep red blood cells (rbc) and application of allogenic skin grafts respectively to test for possible immunodepression in the leukemic cat. Finally, kidneys were to be evaluated by immunofluorescence for deposition of feline globulins suggesting immune complex deposition.

   IgG has been isolated from cat antiserum to sheep rbc.

   IgM separation has been accomplished using preparative ultracentrifugation and molecular sieve chromatography. Pools of saliva and tears from several cats stimulated with pilocarpine have been collected and concentrated for IgA separation and purification.

2. Preleukemic disease and immunosuppression - This investigation was designed to determine the sequential histologic alterations in lymphoid tissues, and bone marrow of preleukemic cats and to correlate these data with alterations in cell-mediated immunity. Twenty specific - pathogen-free (SPF) cats have been inoculated with feline leukemia virus. All cats inoculated with this viral stock can be expected to develop lymphosarcoma by 120 days of age. Twenty uninoculated control SPF cats serve as age-matched controls for the study. Striking thymic atrophy is manifest at 5 weeks of age at which time the mean thymus weight of preleukemic cats is 18% of control. Atrophy of the thymus is associated with a marked deficiency in cell-mediated immunity. FeLV-infected cats evaluated thus far (from 5 to 11 weeks of age) have significantly impaired capacity to reject cutaneous allografts from the same donor. Allograft rejection is still incomplete at 21 days post grafting.

   The onset of lymphosarcoma occurs at 8 to 10 weeks of age and is manifest by appearance of significant numbers of immature lymphoid cells in the bone marrow and by mesenteric lymphadenopathy. Large lymphoid cells accumulate initiatly in the deep cortex and proximal medullary sinuses of the mesenteric lymphodenopathy.

Data collected to date support the working hypotheses that 1) thymic atrophy and impaired cell-mediated immunity precede the onset of feline lymphosarcoma and 2) lymphosarcoma cells may originate in the bone marrow where they can be detected at or before the onset of the characteristic lesions of lymphosarcoma in lymphoid tissues. These manifestations may prove to be important early signs of the disease. They may permit a rapid preliminary diagnosis, therefore accellerating the evaluation of treatments such as vaccination, etc.

3. Vaccine development and evaluation - Objectives and experimental outline-killed virus vaccines prepared against the feline leukemia and sarcoma viruses are being analysed to determine their effectiveness as a means to prevent neoplastic disease in experimentally challenged cats. Results of this study may yield information concerning the feasibility and possible use of oncogenic virus vaccines in other species including man.

   Evaluation of vaccine effectiveness is being determined in vivo by challenge with oncogenic sarcoma virus preparations and in vitro by neutralization of observed feline sarcoma virus cytopathic effects.

Significance to Biomedical Research and the Program of the Institute:

Knowledge as to the hazard associated with virus and infectious nucleic acid transmission and crossing of species barriers is very important in the evaluation of risk where working with tumor virus and their nucleic acids. The development of a proto-type vaccine (feline) against a tumor virus in a random bred animal population will permit one to assess the feasibility of using such prophylactic measures in the control of human malignancy that possibly is induced by virus. The rather high natural incidence of such an etiologically associated disease in the cat makes this a very excellent model, which may be used as a human simulant. Control of this disease in the feline would suggest that it is feasible to accomplish this in man if a human tumor virus of sufficient range were found. Methods of extracting, processing and utilizing antigens from such viruses, which could be certified to be free from any genetically transmittable information would be a significant and very important step in permitting the licensing of such a future vaccine.

Proposed Course: In order to develop a model system to study the susceptibility of a host to concurrent infection and cross infection, the cat model is being developed. We plan to study this animal as one would humans on chemotherapy, in order to determine his capacity for antibody synthesis, response to chemotherapy, enhancement of malignancy by immunosuppression and look at the significance of hormonal imbalance in the development of malignancy. Both cellular and humoral immunologic responses will be investigated.

Date Contract Initiated: June 22, 1965

Current Annual Level: $257,570

NAVAL BIOLOGICAL LABORATORY (FS-57)

Title: Studies of Environmental and Physiological Factors Influencing Virus-Host with Interaction

Contractor's Project Directors: Dr. R. L. Dimmick
Mr. M. A. Chatigny

Project Officers: Dr. A. Hellman
Dr. A. K. Fowler
Mr. W. E. Barkley

Objectives: This contract has three objectives, they are:

1. Virus laboratory hazards evaluation. The objective of this section of the proposal is to evaluate the extent of possible hazards involved in biochemical and biophysical procedures used in virus-tissue culture laboratories.

2. Studies on environmental effects on physical and biological characteristics of viral aerosols. The objective of this section of the proposal is to provide survival data of both "model" and oncogenic viruses as related to environmental parameters (e.g. temperature, RH, RH changes, and trace chemicals - decontaminants) for use in Section 1, and to evaluate the importance of end-spectrum (0.1 to 0.5 mμ) (5 to 15 mμ) particles on virus-host interaction considering both the hazard to humans and animals and the potential for cross contamination.

3. Host-virus interactions. The objective of this section is to evaluate the effect of selected stress situations (physiological as by hormonal imbalance, immunological as by concurrent infection or biochemical, as by exposure to injurious chemical vapors of aerosols) on induction of viral disease or cancer in situ, and to evaluate the role airborne particle size might play in such interactions.

Major Findings:

1. Virus laboratory hazards evaluation. Preliminary data shows that use of tracers other than actual oncogenic virus will yield information equivalent to virus-data, and that use of such tracers will increase productivity.

   We have completed our test of a "Branson" sonic oscillator. Results substantiate the concept that a physical tracer can be effective.

   Initial tests have been made with an angle head centrifuge rotor, at 0 to 5000 rpm. The regular seal was intact and the rotor was filled with a suspension containing 10^8 bacteria/ml. The output was greater than 2.4 x $10^3/ft^3/min$, but there were no particles

collected by an Andersen sampler in the 1 μ size range; the majority were 8 μ or larger. We are using this rotor to simulate the possible function of the zonal while we establish appropriate sampling techniques.

2. Studies on environmental effects on physical and biological characteristics of viral aerosols. Stability of the simian virus SV-40 has been tested at 7 values from 5 to 90% relative humidity (RH) and at 70°F and 90°F. At 70°F there was practically no loss in viability; at 90°F, in the range of 45 to 60% RH less than 0.1% remained viable after 60 minutes.

3. Host-virus interactions. Using tritium labeled estradiol, we have shown in a preliminary experiment that the respiratory dosage of estradiol calculated by atomizer output, air flow rate, and Guyton's formula (breathing rate for mice = 1.25 ml/gm/min) yielded a substantially correct value.

   The effective dilution ratio $\frac{\text{Activity in mouse lung}}{\text{Activity/ml in atomizer}}$ was 12.6×10^{-5}. The calculated dosage was 4.2×10^{-8} gms/mouse; the value calculated by the ratio was 4.7×10^{-8} gms/mouse. This dosage caused an increase in weight of the uterus of ovarectomized mice, acted as a group specific antigen activator, and was not transmitted to cagemates.

Significance to Biomedical Research and the Program of the Institute:

An understanding of the survival and clearance rate for virus aerosol will allow the development of a rational and hopefully less expensive means for control of cross infection. Similarly development of mathematical models will permit the more precise evaluation of facilities, on the basis of risk. Transmission of chemicals that are found in the environment, which have been demonstrated by Hellman and Fowler to induce activation of tumor virogenic markers (g.s. and reverse transcriptase), make an understanding for their potential to activate such markers by the respiratory route very important in the evaluation of risk either as a primary or co-inducing factor.

Proposed Course: We are currently evaluating the output of blendors using fluorescein dye. The aerosolized dye is collected in AGI impingers and measured spectrophotometrically. Additional equipment or techniques to be tested are:

1. Centrifuge
2. Culture shaker
3. Mechanical mixer
4. Dropping liquid on surface
5. Mixing with a pipette
6. Dropping a flask

In instances where aerosol output is too dilute to measure by fluorescein output, we will employ the NBRL microaerofluorometer, supplemented by biological tracers.

At present, test units are being held in the smallest possible space to ensure maximal collection efficiency. We intend to test a limited number of the above in a large volume (room) where ventilation and gross air patterns can be reasonable controlled.

We are concerned that our present nomograph is too complex for convenient use. We must, however, ensure that the range of each variable includes all possible operating conditions. We will investigate the question of whether, in a given case, the assumption of a uniform concentration in a room compared to the assumption of limited spaces with unequal concentrations will yield exposures that are equivalent within desired limits of accuracy.

A thermal precipitator is being constructed. It will be used to collect particles in the sub-micron range.

Date Contract Initiated: March 1, 1971

Current Annual Level: $100,000

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NIH-71-2348)

Title: Study of Latent Virus Infection and Transmission

Contractor's Project Director: Dr. S. S. Kalter

Project Officer (NCI): Dr. Alfred Hellman

Objectives: It is the purpose of this contract to study various aspects of the role of latent virus infections of laboratory animals and cultures of their organs as a source of contamination of experimental materials, laboratory personnel and vaccines. As a part of these studies the rate of inactivation of virus infectivity and oncogenicity by heat and ultraviolet light will be determined for several viruses in the extra- and intracellular state. The viruses selected for study are of interest because of their demonstrated oncogenic potential, ability to maintain a persistent, inapparent infection of cell cultures and the physical state of their genome.

The possible transmission of cancer by inhalation of tumor cells will be studied in mice and hamsters by inoculation via the intranasal route with tumor cell suspensions and aerosols.

The role of antiserum in establishing persistent infections will be studied.

Major Findings: Studies on the intracellular inactivation of H. hominis types 1 and 2 at 4°C, RT, 37° and 56° have continued. During the last report period intracellular (Vero) virus had persisted at 4° for 16 days. This experiment was continued to 42 days with little, or no, drop in titer for either virus. Data collected on thermal and U.V. inactivation of HSV 1 and 2 indicated intracellular virus was inactivated thermally more slowly than extracellular virus, but U.V. inactivation occurs about the same rate in both states. Emperical observations suggest cell clumping, pH, medium composition and cell type are factors which influence reproducibility of results from sample and laboratory to laboratory. U.V. irradiated HSV 1 and 2 with infectivity destroyed have been inoculated into hamster embryo monolayers in a preliminary attempt to induce cellular transformation. After 7 weeks no changes have been noted. C_3H mouse tumor being carried by inoculation of the tumor cell suspension into the leg muscle of C_3H mice has been studied for its ability to induce tumors by the intranasal route. A suspension of 10^6, 10^5 and 10^4 cells per 0.02 ml was prepared. Approximately 0.01 ml of this suspension was inoculated into 2 week old C_3H mice by dropping onto the external nares and allowing it to be inhaled. All mice became ill and died or were sacrificed in a moribund state by the end of 3 weeks. Lung material from these animals was inoculated intramuscularly into other C_3H mice for passage and processed

for histopathology. Tumors were not grossly obvious on the lungs. Histopathological observations are not completed.

Four perinatal baboons were inoculated with Feline Leukemia Virus (Rickard Strain) received from the Project Officer by way of Virgo Reagents. No disease was noted in any of these animals and they were subsequently challenged with Tween 80 - Ether treated Feline Leukemia Virus antigen by the subcutaneous and intramuscular route. No viral antibodies have been detected in sera from these animals.

Significance to Biomedical Research and the Program of the Institute:

An understanding of whether latent virus is present in certain animal cell cultures permits one to evaluate the feasibility utilizing such a culture for propogation of virus for the development of vaccines. Similarly an understanding of how to make latent virus express itself will shed light into the whole question of transmission of genetic information and what factors stimulate the packaging of such information into whole virus. A knowledge of the physical factors necessary for activation and inactivation of tumor virus will provide a more rational approach to investigator protection.

Proposed Course: More detailed studies of the HSV 1 and 2 U.V. inactivation are planned to determine the influence of cell culture and state of intracellular virus on rate of inactivation. Thermal inactivation studies will be completed. Feline tissue explants will be started to determine latent virus in SPF cats. Respiratory inoculation with C_3H tumor cells and virus particles will be studied in more detail. A collaborative effort using primates and endocrine activation of tumor virus expression is being initiated as yet another method for detection of non-expressed virus information.

Date Contract Initiated: June 3, 1971

Current Annual Level: $57,400

UNIVERSITY OF MINNESOTA (NIH-NCI-72-2066)

Title: Development of Short Courses on the Principles of Biohazard and Injury Control for the Biomedical Laboratory

Contractor's Project Director: Prof. G.S. Michaelsen

Project Officer (NCI): Mr. W. Emmett Barkley

Objectives:

1. The University of Minnesota is responsible for the development of short courses on contamination control, biological, chemical and physical safety for biomedical laboratory personnel. Detailed training manuals, including lecture notes, literature references, tables, figures and laboratory exercises are to be prepared.

2. Four courses are to be presented annually to scientific personnel engaged in oncogenic virus research.

Major Findings: A course schedule, including approximately 18 topics and 3 laboratory sessions, has been developed. Lecturers for these topics have been selected.

The first course is scheduled for June 12-16, 1972 at the University of Minnesota. The remaining three course presentations will occur during the second half of the contract year. The course dates and location for the remaining three presentations have been selected and confirmed.

Significance to Biomedical Research and the Program of the Institute: This contract will provide an opportunity to scientific investigators and laboratory technicians engaged in cancer virus research to learn the fundamentals of laboratory safety and environmental

control including the correct usage of biological safety hoods, the adequacy of protective clothing and other personal protective devices, the role of disinfection and sterilization techniques and the principles of biological, physical, chemical and radiological hazard control.

In addition, course materials developed under this contract will be made available to the safety office of the Center for Disease Control, Atlanta, Georgia and to other appropriate safety groups in the biomedical community. These materials will make it possible for safety organizations to provide excellent safety training opportunities for all biomedical research personnel.

Proposed Course: The contract was initiated in December, 1971. The proposed course is to present four courses during the contract year to investigators and senior technicians in the SVCP. Two courses will be presented in Minneapolis and two at other locations corresponding to high density SVCP contract areas.

Date Contract Initiated: December 10, 1971

Current Annual Level: $105,000

BREAST CANCER VIRUS SEGMENT
Dr. W. Ray Bryan, OSD, Etiology, NCI, Chairman
Dr. Robert Depue, OSD, Etiology, NCI, Vice Chairman
Dr. Harry J. Clausen, OSD, Etiology, Executive Secretary

GEORGETOWN UNIVERSITY SCHOOL OF MEDICINE (PH43-NCI-E-65-53)

Title: Human Breast Cancer Studies

Contractor's Project Director: Dr. William F. Feller

Project Officer (NCI): Dr. W. Ray Bryan

Objectives:

1. Continuation of studies on the association of virus-like particles in human milk, with human breast cancer.

2. Tissue culture attempts to propagate virus-like particles observed in human milk and breast cancer cells.

3. Attempts to induce mature virus production in established tissue culture lines of human cancers, particularly breast cancers.

Major Findings:

The most important result was the successful induction of virus production in human cancer cells in tissue culture by the method recently introduced by Rowe and associates for the activation of leukemia virus in mouse cells, namely by treatment with IUDR. The additional finding was made that treatment with DMSO following IUDR enhanced the reaction by a factor of 10 or more. The human cell cultures involved were established lines of a rhabdomyosarcoma, and of a metastatic lesion in a node of a patient with lung cancer. Virus particles were detected by electron microscopy. Whereas the particles observed in the lung cancer cells budded from cell membranes and in every way resembled the known C-type oncogenic RNA viruses, those of the rhabdomyosarcoma budded from the endoplasmic reticulum and were intracisternal rather than extracellular. Most of the particles resembled A-type particles but many of them had dense nucleoids and resembled C-type particles except for their intracisternal location. Further studies are in progress to rule out contamination of the cultures with some murine virus. If the latter are ruled out, this will represent the first successful induction of a human oncorna virus in an *in vitro* system.

Efforts to induce particle production in human breast cancer cultures by similar procedures have not been successful thus far.

In the studies on human milk, the new biochemical method of Spiegelman and Schlom for the detection of oncorna-type viruses has been introduced to replace the more time consuming and much less sensitive method of electron

microscopy. It involves the simultaneous test for 70 S RNA and reverse transcriptase. This method is now being used in the monitoring for selecting positive milks from which sufficient virus-type material can be recovered to support investigation directed toward propagating the agent in tissue culture.

Significance to Biomedical Research and the Program of the Institute:

This contractor was one of three initially selected to investigate the possible association of viruses with human breast cancer, and, in collaborative studies involving electron microscopic monitoring by another contractor (67-1176), was the first to show that particles resembling the oncogenic RNA viruses are present in milk of women who have had breast cancer.

This observation led to an expansion of the effort on breast cancer under the SVCP, and the studies under this contract are essential for determining whether the candidate agent being observed is actually virus, and related to the causation of the human disease.

Proposed Course:

The effort toward the objectives described will be continued at the current level.

Date Contract Initiated: 11/19/64

Current Annual Level: $171,400

HOWARD UNIVERSITY SCHOOL OF MEDICINE (NIH-NCI-E-70-2178)

Title: Immunological Studies on Human Breast Cancers and Other Neoplasms

Contractor's Project Director: Dr. Michael V. Viola

Project Officer (NCI): Dr. Ronald B. Herberman

Objectives:

1. Procurement of human specimens for use by SVCP participants as well as for research conducted in the contractor's institution.

   a. Selected specimens of breast and other cancers procured at operation or biopsy.

   b. Sera from patients with breast and other cancers, and from patients with non-neoplastic diseases for use as controls.

2. Establishment and characterization of tissue culture lines from selected cancer patients, particularly breast cancer.

3. Immunological studies of established cancer cell lines and of patients with breast cancer and other neoplasms.

Major Findings:

1. Procurement of specimens

   a. The following numbers of specimens have been distributed to 11 intramural and extramural participants of the SVCP.

Breast carcinomas	25
Fibrosarcomas	2
Gastrointestinal carcinomas	5
Lung carcinomas	2
Vulva carcinomas	3
Malignant effusions (breast cancer)	2
Lymphocyte preparations from Hodgkins patients	2
Lymphocyte preparation from breast cancer patient	1
Lymphocyte preparation from patient with CLL	1

   b.

Sera from breast cancer patients and matched controls	35
Sera from Hodgkins patients and matched controls	50
Selected cancer sera and controls	54
Large plasma specimens with high antibody titers against Be Lev cell line	2

2. Tissue culture studies:

   A cell line (W-1) has been established from a malignant pleural effusion from a patient with disseminated breast cancer. Using the simultaneous detection method, high molecular weight RNA (70 S) and reverse transcriptase were found in the concentrated supernatant from this cell line. It is now being further characterized, and cloned, for use in immunological and virological studies on human breast cancer.

3. Immunological studies of patients:

   Thirty-five patients with breast cancer and a similar number of controls have been studied with respect to lymphocyte response to various dilutions of PHA. Response to PHA decreased in disseminated breast cancer. The decrease was found not to be due to serum factor, and an attempt to correlate lymphocyte response to relative proportions of B and T lymphocytes is now in progress.

   Personnel from the contractor's laboratory are now being trained in the Project Officer's laboratory at NCI in several new immunological techniques to be employed in collaborative studies on breast cancer and other patients at the contractor's institution.

Significance to Biomedical Research and the Program of the Institute:

The NCI does not currently have an intramural program on the study of human breast cancer, and this university hospital in the Washington, D.C. area provides the important needs of (1) breast cancer patients for study by intramural scientists, in collaboration with the contractor; and (2) fresh cancer tissue, lymphocytes, and other specimens for tissue culture, immunological and virological studies both by intramural scientists and other SVCP participants.

Proposed Course:

Personnel from the contractor's laboratory are being trained in the Project Officer's laboratory at NCI for expanded immunological studies of cancer patients, particularly those with breast and colon cancers. An increase of about $6,000 in funding is projected.

Date Contract Initiated: 4/27/70

Current Annual Level: $49,729

INSTITUTE FOR MEDICAL RESEARCH (PH43-NCI-E-68-1000)

Title: Studies of Human Milk and Mammary Tumors

Contractor's Project Director: Dr. Dan H. Moore

Project Officer (NCI): Dr. W. Ray Bryan

Objectives:

To explore human milk and breast cancer tissue in search of a virus that might be etiologically related to the disease.

Major Findings:

As previously reported, important leads suggesting the possible involvement of a virus in human breast cancer were obtained in exploratory studies, namely: (1) the finding of virus-like particles resembling the oncogenic RNA viruses in some milk specimens from women belonging to high-breast-cancer families, and (2) the finding of reverse transcriptase activity in such particle-positive milks (initially collaborative with contract #70-2040).

A multidisciplinary effort was initiated at the beginning of this contract year to follow-up these leads on the human problem and to use the mouse mammary tumor virus (MTV) system to guide the human studies.

A biochemical laboratory was added and 25 human milk specimens studied thus far show that about one-third of them were positive for reverse transcriptase, confirming the earlier exploratory results. In other biochemical studies the

five major proteins of purified mouse MTV recently reported by Nowinski have been confirmed, and an additional protein has been identified in the 69,000-110,000 molecular weight range which is thought to be the enzyme, reverse transcriptase.

New techniques developed in the expanded tissue culture laboratory have resulted in the successful explanation of 71 human breast cancers. Forty-five have been investigated thus far by negative staining and thin section electron microscopy. Spiked particles resembling the MTV have been found in 3, and smooth surfaced particles resembling C-type virus have been found in 24. A third type of particle with shorter spikes than the MTV was found in 8 cultures. Double blind studies are in progress to determine the association of reverse transcriptase with type of particle. A total of 172 milk specimens have been collected for this purpose from women of high-breast-cancer risk and control populations.

Neutralization tests have been initiated on 60 human sera (from breast cancer patients and controls) to determine whether they reduce the activity of MTV in test mice. Other immunological studies underway include: (1) the immunization of rabbits with high-speed pellets from human milk containing high reverse transcriptase activity and a high number of virus-like particles, as a source of specific antibody against the candidate human particles; and (2) the immunization of test mice with such human candidate virus to determine whether it will protect against later challenge with the mouse MTV.

Significance to Biomedical Research and the Program of the Institute:

This was one of three projects initially set up to determine whether sufficient evidence could be found for an association of virus with human breast cancer to justify a broader formal program on the virus approach to the etiology of the human disease. The results of studies under this and other related contracts (see also PH43-NCI-E-65-53 and PH43-NCI-E-67-1176) led to an expansion of programmed activities on breast cancer under this as well as the other contracts.

Proposed Course:

This multidisciplinary effort will continue at its current level for further electron microscopic, biochemical, tissue culture, and immunological investigations of the candidate human virus in milk and breast cancer tissue.

Date Contract Initiated: 6/28/68

Current Annual Level: $402,000

MASON RESEARCH INSTITUTE (NIH-NCI-E-70-2204)

Title: Hormonal Influences on the Induction of Breast Cancer in Specific Virus Infected Animals

Contractor's Project Director: Dr. Marcus M. Mason

Contractor's Principal Investigator: Dr. Arthur E. Bogden

Project Officer (NCI): Dr. W. Ray Bryan

Objectives:

(1) By studies of the hormone profiles of blood and urine of experimental animals during normal physiological cycles and during different regimens of hormone treatment, to determine the hormone dosages, combinations, and sequences to be tested in the virus-hormone co-carcinogenesis studies of objective (2).

(2) To determine whether candidate viruses isolated from animal breast cancers are capable of inducing breast cancers in their natural hosts and/or animals of other species, using appropriate hormone stimulation of the hormone-dependent target tissues (mammary glands). Candidate viruses currently under investigation are: (a) the Mason-Pfizer Monkey Virus (M-PMV) isolated from a breast cancer of a Rhesus monkey at the contractor's institution; and (b) the R-35 virus isolated from a transplant of a spontaneous breast cancer of a Sprague-Dawley rat, also at the contractor's institution.

Major Findings:

M-PM Virus

The appropriate regimens for hormonal treatment (hormones, dosages, sequences and combinations) have been worked out in female Rhesus monkeys for (1) producing hypertrophy of the breasts with hyperplasia of the ductular and alveolar epithelium, and (2) for inducing and maintaining lactation of such stimulated mammary glands. These regimens are now being applied in two types of investigations: (1) Hormone treatment of female monkeys inoculated as newborns with M-PMV. Treatment is begun when they reach 18 months of age and will be continued for prolonged periods as a co-factor in breast tumor induction. The animals will be observed for two to three years for the appearance of breast cancers. (2) Inoculation of sexually mature, hormone treated, female Rhesus monkeys with M-PMV directly into the hyperplastic ductules and alveolar tissues to determine their susceptibility to infection with M-PMV. Infectivity is currently being tested by inducing lactation and examining milk for C-type M-PMV particles, reverse transcriptase activity (collaborative with others), and the presence of specific viral antigens. These studies are at an early stage and sufficient results are not available for evaluation at this time.

R-35 Virus

Until recently, no rapid in vitro method was available for determining the biological activity of this virus, and the fact was not previously known that its biological potency is greatly diminished by freezing and storage.

In experiments initiated with frozen virus, before this information became available, the following results have been obtained:

A total of 5 female Sprague-Dawley rats inoculated as newborns with the R-35 virus have developed mammary adenocarcinomas among a total of 98 inoculated; giving an overall incidence of 5.1%. The time of appearance varied from 4 to 14 months, all but 1 appearing in rats less than one year of age. No tumors of this type were observed among 197 uninoculated controls within the same experiments.

All of the mammary adenocarcinomas occurred in rats inoculated with 3 of the 9 different lots of frozen virus used in the studies, with the following frequencies: 2/36 (5.6%), 2/16 (12.5%), and 1/20 (5.0%). No tumors of this type occurred among animals inoculated with the other 6 lots of frozen virus.

Another type of tumor, fibroadenoma, known to occur spontaneously in older animals of this strain has been observed in two uninoculated control animals at approximately 11 months of age.

Although the small number of mammary tumors observed does not represent definitive evidence that the R-35 virus is oncogenic for rat mammary tissue, the results lend strong encouragement to this possibility. These studies are now being repeated with fresh virus inoculated on the same day as it is prepared, which should have much stronger biological activity.

Significance to Biomedical Research and the Program of the Institute:

At the present time, breast cancer is known to be caused by a virus in only one animal species, the mouse. This project, involving biological testing of candidate viruses for oncogenicity, is part of a broader program activity for determining whether viruses are related to breast cancer in animals other than mice. Such animal studies are necessary for developing technology and approaches to the study of the human breast cancer virus problem.

Proposed Course:

The experiments under way will be continued until the planned numbers of experimental animals have been inoculated with virus (50 monkeys, 2000 rats, and several hundred each of mice and hamsters, all inoculated as newborns). Observations for tumor induction will be continued for the lifetimes of the rodents, and for 2-3 years in the case of monkeys. If oncogenicity is demonstrated for any candidate virus, systematic follow-up studies will be introduced.

Date Contract Initiated: 6/9/70

Current Annual Level: $265,000

MEDICAL COLLEGE OF WISCONSIN (PH43-NCI-E-68-1010)

Title: Hormone Effects on Virus Particle Activity in Breast Cancer

Contractor's Project Director: Dr. Ronald Pattillo

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives:

To study the effects of human hormones on possible oncogenic virus production in human breast cancers in vitro. The hormones to be studied include the lactogenic hormone and biologically active estrogenic and progestational steroids.

Major Findings:

A series of breast tumors have been studied in the initial experiments to define culture conditions, optimum type of tumor-hormonal responsiveness and potential for continued growth in culture. Encouraging new leads that may have relationship to the recently reported identification of virus particle production within epithelial "domes" from mouse mammary tumors have been observed in one of the human tumor cultures. Attempts to subculture these "focus" appearing cell aggregates have not as yet been successful. However, these experiments suggest a possible parallel between mouse mammary tumor behavior in vitro and the human tumor. The culture medium is modified by conditioning with the hormone secreting trophoblast cells in vitro. This medium also contains polyvinylpyrrolidine which favors the growth of tumor cells in suspension. Several of the tumors show epithelial cells which appear morphologically similar to breast cancer cells. In the cultures, to date, insufficient cells are available for testing for functional markers such as casein or the production of B-particles.

Significance to Biomedical Research and the Program of the Institute:

The contractor has recently established a multipotential human hormone synthesizing trophoblastic cell system which is used in co-cultivating experiments under a variety of conditions aimed at providing suitable hormone conditions for human breast cancer cell propagation. These studies may provide an important insight into the hormonal effects on virus activity in human breast cancer.

Proposed Course:

The effort toward the objectives stated will be continued at the current level.

Date Contract Initiated: 9/19/63

Current Annual Level: $111,635

MEMORIAL HOSPITAL (NIH-NCI-E-71-2194)

Title: Procurement of Human Serum Specimens from Defined Population Groups for Immuno-epidemiological Studies

Contractor's Project Director: Dr. Herbert F. Oettgen

Project Officer (NCI): Dr. Harry J. Clausen

Objectives:

Procurement of serum specimens from the following defined population groups as a part of a collaborative effort to determine whether candidate viruses isolated from human or animal sources are related etiologically to human breast cancer.

Basic defined population: Women entering Memorial Hospital, New York City, for first diagnosis of any breast disease.

Test group: Women whose lesions prove to be malignant as determined by biopsy.

Control groups:

a. Women whose lesions are found on biopsy to be benign proliferative reactions or reactions suspected as being pre-neoplastic in nature.

b. Women whose lesions are considered to be unrelated to neoplasia.

Major Findings:

This contract was negotiated 6/23/71, but there was a 6-month delay in activating it because laboratory space to be occupied did not become available until January. The previous occupants were unable to vacate because their new quarters could not be made ready for occupancy due to a strike in New York City. Since there was a delay in starting the activities under this contract the serum specimen procurement did not start until April.

Significance to Biomedical Research and the Program of the Institute:

Since viral agents suspected of causing cancer in man cannot be tested directly in human subjects, it is necessary to establish etiological relationship indirectly through immuno-epidemiological studies. This contract is for procuring the epidemiologically defined bank of serum specimens essential to the determination of whether antibodies against suspect viruses occur with higher frequency, and in larger amounts, in sera of women with breast cancer as compared with appropriate controls.

Proposed Course:

It is anticipated that a sufficiently large bank of specimens for the proposed sero-epidemiological studies can be acquired under this contract within a period of 2 to 3 years.

Date Contract Initiated: 6/23/71

Current Annual Level: $46,340

MICHIGAN CANCER FOUNDATION (NIH-NCI-E-71-2421)

Title: Studies in High Breast Cancer Families

Contractor's Project Director: Dr. Michael J. Brennan

Project Officer (NCI): Dr. Harry J. Clausen

Objectives:

The first year of this contract was a feasibility study to determine:

(a) If large quantities of human milk containing sufficient virus-like particles could be secured through monitoring large numbers of milk specimens taken from lactating patients on obstetrical services in the Detroit metropolitan area and to establish demographic genetic characterizations of these patients' families so as to determine the strength of the genetic or nongenetic factors which might be associated with virus-like particle secretion in the milk.

(b) Whether electron microscopy, through negative staining procedures, represented an adequate method of monitoring milk specimens, and the selection of those which contained relatively large amounts of virus-like particles.

Major Findings:

Collections of milk samples are underway at 31 of the 43 obstetrical services in the area and currently milk specimens are being obtained from some 150 nursing women per month. Objective information on the causes of death and neoplastic disease experience is now complete for 70 families and partially completed for another 100 families. Therefore, the operational base upon which large-scale studies of a fully demographic spectrum of young nursing mothers has been established and the characteristics can now be developed in relation to breast cancer etiology and development.

Some 335 milk specimens have been shipped to NCI as well as other investigators involved in the Special Virus Cancer Program. To date few, if any, fully typical B particles have been demonstrated. By means of negative staining electron microscopy milk specimens have been found which contain particles of a virus-like appearance but not fully meeting the criteria for

"B" particles. It is felt that particles are being destroyed by a milk component and further work on particle preservation is being continued.

Significance to Biomedical Research and the Program of the Institute:

At this time, breast cancer is known to be caused by a virus in only one animal species, the mouse. Therefore, studies based on leads from this animal species have been conducted to determine if evidence might be obtained for an association of viruses with human mammary cancer. The "clustering" of breast cancer within family groups and its similarity to that observed for mouse mammary cancer before the development of inbred mouse strains has suggested that a virus might also be involved in human breast cancer. Efforts to obtain additional evidence on a basis of which virus-like particles in human milk might be demonstrated to be actual virus are in progress.

Proposed Course:

Since large scale monitoring of human milk specimens with negative staining EM alone has not proven satisfactory, it is proposed that the primary monitoring will be done in the future with new biochemical techniques that have recently become available, namely the simultaneous test for 70 S RNA and reverse transcriptase. EM will be used only for confirmation on those milk specimens that prove to be positive for reverse transcriptase and 70 S RNA. These monitoring and investigative procedures on milk samples will be conducted in the contractor's laboratory as well as the study of medically indicated biopsy material and lactating mammary tissue from collaborating donors. Furthermore, studies on methods for preserving the morphological integrity and enzyme activity of the virus-like particles in milk samples will be continued.

Date Contract Initiated: 6/20/71

Current Annual Level: $119,000

PFIZER INC. (PH43-NCI-E-67-1176)

Title: Virological Studies of Human and Animal Breast Cancers

Contractor's Project Director: Dr. K. E. Jensen

Project Officer (NCI): Dr. W. Ray Bryan

Objectives:

1. Electron Microscopic Studies

   An electron microscopic laboratory with professional and supporting staff is operated for two purposes: (a) The search for viruses in human and animal breast cancers, in collaboration with other SVCP

participants not having this capacity; and (b) EM monitoring and research at the contractor's institution associated with virological studies on new candidate viruses isolated from breast cancer (see objective 2).

2. Virological studies directed toward the further characterization of new candidate breast cancer viruses, and the development of new animal model systems for guiding research on the human problem.

Major Findings:

Previous studies involving the EM collaboration of this contract have resulted in the finding of virus-like particles resembling known oncogenic RNA viruses in the milk of women who have had breast cancer, and the discovery of two new candidate viruses isolated from breast cancers of animals--the Mason-Pfizer Monkey Virus (M-PMV) and the R-35 rat virus (R-35V). The successful propagation of the latter two animal viruses, in collaboration with another contract at the contractor's institution (70-2080), led to the supplementation of this contract during the current year to provide a facility specifically for the study of breast cancer viruses, and to carry out systematic studies on the R-35 and M-PM viruses.

The following additional results have been obtained:

R-35V

This virus has been found to infect and transform normal lactating rat mammary gland cells in tissue culture. The reaction appears to be specific for mammary tissues since other types of rat cells (e.g. spleen, thymus, bone marrow, kidney) were neither infected nor transformed. An in vitro assay method based upon focal transformation of the susceptible cells has been developed and is now being used in systematic virological studies.

Evidence that the R-35 virus is oncogenic is represented by the fact that transplants of foci transformed in vitro to weanling rats grow as highly malignant tumors. The latter contain both carcinomatous and sarcomatous components.

The virus has been found to contain gs 3 antigen but not the gs 1 antigen of any of the mouse oncogenic viruses.

M-PMV

An immunofluorescent end-point assay has been developed for the in vitro assay of the M-PMV in NC-37 cells (of human origin) which it has been found to infect.

Specific antigens of the M-PMV have been observed in 2 of 5 normal Rhesus monkey embryos tested, indicating that this virus might be indigenous in this species.

Immunological studies have shown that the M-PMV virion contains five distinct antigens, three associated with the coat and two with the core, or nucleoid.

Significance to Biomedical Research and the Program of the Institute:

The contractor's EM laboratory continues to be a key participant in a broad collaborative program in the search for viruses related to human and animal breast cancers.

The further research and development on the two new candidate animal breast cancer viruses in the contractor's laboratory is essential to the application of these new models to studies of the possible viral etiology of human breast cancer.

Proposed Course:

The activities described will be continued at the current level until the determination is made whether these candidate animal viruses are etiological agents of breast cancer of the types from which they were isolated.

Date Contract Initiated: 6/28/67

Current Annual Level: $328,000

TEL AVIV UNIVERSITY (NIH-NCI-E-72-3237)

Title: Isolation, Purification and Propagation of Virus-like Particles from Human Milk in Israel

Contractor's Project Director: Dr. Jafa Keydar

Project Officer (NCI): Dr. Timothy E. O'Connor

Objectives:

Purification of candidate virus from various milk samples obtained from women of designated high-breast-cancer-risk populations in Israel and assay for RNA-instructed DNA polymerase as well as attempts to grow the milk "virus" in human tissue cultures.

Major Findings:

This contract was initiated late in the fiscal year (March, 1972) and although work toward the stated objectives is fully under way, it is too early to assess the significance of apparent progress already being made.

Significance to Biomedical Research and the Program of the Institute:

Israel is a particularly favorable setting for an investigation of this type since high-risk donors of milk are easy to identify and line-up for milk studies

well in advance of delivery, because of the following reasons: (a) Israel has a mandatory national cancer registry and the occurrence of breast cancers within families can be readily determined, (b) Israel has a national health plan which includes maternity cases who report for care early in pregnancy, allowing ample time for surveys for cancer history, (c) most women deliver in hospitals thus simplifying the logistics of milk procurement, and (d) most importantly, there are several ethnic groups within Israel having breast cancer risks among the highest known.

The evidence of virus-like material in human milk obtained in other SVCP projects makes it essential to propagate this candidate agent for further characterization and determination of its association with human breast cancer. This contractor has unique advantages for pursuing these goals.

Proposed Course:

The project will be continued for at least two years at essentially the same level.

Date Contract Initiated: 3/23/72

Current Annual Level: $71,750

UNIVERSITY OF CALIFORNIA, DAVIS, CALIF. (NIH-NCI-E-72-2080)

Title: In vitro cultivation of human and mouse mammary tumor viruses

Contractor's Project Director: Dr. Robert D. Cardiff

Project Officer (NCI): Dr. Robert H. Depue

Objectives:

1. To study human breast tissue under various experimental conditions developed from the mouse mammary model for the presence of possible human mammary tumor viruses.

2. To study the production of mouse mammary tumor virus (MTV) in vitro in order to develop concepts, techniques, and reagents that might be applicable to larger scale production of this virus and of potential human breast cancer viruses.

Major Findings:

This contract (negotiated February 1, 1972) is still in the early stages of implementation and although tissue culture studies on both human and mouse tissues are in progress, no experiments have yet been completed.

Significance to Biomedical Research and the Program of the Institute:

The finding of virus-like particles as well as 70 S RNA and reverse transcriptase in human milk and breast cancer tissue cultures by several investigators within the SVCP strongly suggest that a virus may be associated with human breast cancer. If so, it will be necessary to produce such virus in large quantities in vitro to further characterize it and determine its relation to the human disease. But this problem, in vitro cultivation, has not yet been solved for the mouse MTV.

This contract is for the development of knowledge and technology for in vitro cultivation and large scale production of the MTV, with simultaneous application of progress to attempts to cultivate the human candidate virus.

Proposed Course: No change in initial contract.

Date Contract Initiated: 2/1/72

Current Annual Level: $99,376

SPECIAL VIRUS CANCER PROGRAM
ETIOLOGY AREA, NCI
Fiscal Year 1972

DEVELOPMENTAL RESEARCH PROGRAM SEGMENT

Dr. Robert A. Manaker, Acting Chief, VBB, Etiology, Chairman
Dr. Michael A. Chirigos, Associate Chief, VBB, Etiology, Vice Chairman

AICHI CANCER CENTER (NIH-69-96)

Title: Virus Rescue Studies in Human Leukemia/Lymphoma Cell Lines

Contractor's Project Director: Dr. Yohei Ito

Project Officer (NCI): Dr. Charles W. Boone

Objectives: (1) To investiage the possible role of Herpes type virus and C-type viruses as etiological agents of human neoplasia, involving the establishment and investigation of numerous human suspension and monolayer cell lines by electron microscopy, immunofluorescence, and other techniques. (2) To make available to the Special Virus Cancer Program human embryonic tissues and high-titer anti-herpes human antiserum.

Major Findings: Nineteen suspension culture lines and about 80 monolayer culture lines were established from tissues of human patients with leukemia, Hodgkin's disease, lymphosarcoma and nasopharyngeal carcinoma. EB virus was identified in the suspension culture lines but was absent in monolayer cell cultures. No C-type viruses were found in any culture.

Over 700 sera from patients with different neoplasms were examined for antibody titers against EBV. Only those with Burkitt tumor, infectious mononucleosis, and nasopharyngeal carcinoma were found to possess high antibody titers.

Significance to Biomedical Research and the Program of the Institute: Since this project is located in the Far East, comparative data acquired in Orientals complements observations made on Caucasian populations. The Principal Investigator has verified observations made in this country and in Europe on the distribution of antibody to Epstein-Barr virus in different neoplastic diseases, and is also engaged in studies on tumors in Orientals which are relatively rare in this country.

Proposed Course: The contractor will emphasize ongoing studies related to the determination of viral reverse transcriptate activity and virus-related nucleic acids in human mammary carcinoma.

Date Contract Initiated: May 2, 1969

Current Annual Level: $40,000

BAYLOR COLLEGE OF MEDICINE (PH43-68-678)

Title: Studies on Viruses as Related to Cancer

Contractor's Project Director: Dr. Joseph Melnick

Project Officers (NCI): Dr. Robert A. Manaker
Dr. Michael A. Chirigos

Objectives: To determine the relationship of viruses to selected neoplasias and their significance in the neoplastic process.

Major Findings: The application of the Cr 51 release cytotoxicity test has demonstrated that the sera of leukemic children contain low levels of antibody reactive with their autologous peripheral blood leukocytes taken during relapse but not to their leukocytes taken during remission. Preliminary studies suggest that cytotoxicity tests may permit specific detection of herpesvirus type 2 antibodies in sera absorbed with cells infected with herpesvirus type 1. The method may be of value in studies on the relationship of HSV-2 to cervical carcinoma. Present testing procedures show the incidence of antibodies to HSV-2 in Caucasian and Negro women in the Houston area to be more than twice that in control women of similar ages and social levels.

Comparative studies on HSV-1 and HSV-2 showed a 70% homology between their DNAs. Recombination experiments on temperature sensitive (ts) mutants of HSV-1 falling into 8 complementation groups revealed recombination frequencies of up to 50 percent with complementing mutant pairs. Non-complementing pairs either failed to recombine or recombined in low frequencies. A defect in some ts mutants in ability to induce synthesis of thymidine kinase was independent of the ts defect. At the non-permissive temperature, one HSV-1 ts mutant accumulates a polypeptide (C-3). This offers the possibility of determining the role of this polypeptide in herpesvirus replication. Thirteen ts mutants of HSV-2 are under study.

Seventeen papers were published during the past contract year on research conducted under this contract.

Significance to Biomedical Research and to the Program of the Institute: This contractor provides a progressive program of research to determine possible etiological relationships of viruses to human cancer. Evidence of possible relationships has been sought by the study of the immune responses of patients and their associates to tumor antigens. Important contributions to Program have been made by the contractor's investigations on genital herpesvirus type 2 associations with malignant carcinoma of the cervix. The importance of herpesviruses in neoplastic processes is evident from the recognition of lymphoproliferative diseases induced by herpesvirus infections as well as a strong association with the development of carcinoma. This contract provides for fundamental studies on herpesviruses which may be invaluable as the role of these agents in precipitating oncogenic processes is further defined.

Proposed Course: The studies relating to childhood leukemia and intensive studies on herpesviruses as agents of apparent importance in oncogenesis will continue.

Date Contract Initiated: June 27, 1963

Current Annual Level: $754,000

COLUMBIA UNIVERSITY (NIH-70-2049)

Title: Replication of Oncogenic RNA Viruses and its Relation to Human Cancer

Contractor's Project Director: Dr. Sol Spiegelman

Project Officers (NCI): Dr. Timothy O'Connor
Dr. Robert A. Manaker

Objectives: To study RNA tumor viruses at molecular levels to elucidate mechanisms whereby they induce neoplastic transformation of cells and to apply this knowledge to the determination of viral relationships in human neoplasms and ultimately to the selection of measures for control of the neoplastic process.

Major Findings: The RNA-dependent DNA polymerase activity was found in the virions of eleven different groups of RNA viruses that cause leukemias or sarcomas, or have been associated with mammary tumors, in different animal species. A DNA-instructed polymerase activity was also found in the virions of all oncogenic viruses examined. By using synthetic polynucleotide duplexes, it was established that oncogenic viruses contain DNA polymerase activities directed by single- and double-stranded RNA, double-stranded DNA, and DNA-RNA hybrids. These activities were shown to be exhibited by a single enzyme. Avian myeloblastosis virus (AMV) reverse transcriptase gave greater dT-primed synthesis with poly rA than with poly dA strands. This appeared to be a means of distinguishing this enzyme from the normal cellular polymerases. By this criterion, enzymic activity was detected in over 120 leukemic buffy coat specimens and in none of 70 buffy coats from normal patients nor in leukoproliferations in diseases other than leukemia. Similar polymerase activity was found in embryonic tissues of animals and man. The highest activities were detected in tissues obtained early in embryogenesis. Further experience showed that every proliferating cell has an elevated response to a dC:dG template, but not all respond to dT:rA and rA:rU.

The purified reverse transcriptase from AMV was shown to consist of two subunits of 110,000 and 69,000 molecular weight respectively, and to be free of ribonuclease and DNA endonuclease activity.

The search for enzymatic evidence of oncogenic RNA viruses in human milk, plasma or tissue is complicated by the low viral content in the presence

of large amounts of enzymatically active debris. A procedure was developed whereby the presence of reverse transcriptase and 70S RNA could be demonstrated in such specimens. The method demonstrated that particles detected in human milk possess these two features characteristic of RNA tumor viruses.

Labeled DNA complementary to oncogenic virus RNA provided a probe to search for RNA in tumor tissues homologous to nucleotide sequences in the viral RNA. RNA extracted from the polysome fraction of 19 of 29 specimens of human mammary carcinoma tissue hybridized with the DNA probe prepared from mouse mammary tumor virus (MTV) RNA but not with DNA complementary to AMV or Rauscher mouse leukemia virus (RLV). No significant hybridization was detected between MTV DNA and polysome RNA extracted from normal adult or benign breast tumor tissue. Further study showed RNA nucleotide sequences homologous to RLV RNA but not to AMV RNA or MTV RNA to be present in the polysome fraction of human leukemic white blood cells and of human sarcomas. No such RNA was recovered from control tissues.

Ten recent publications appeared or are in press describing the observations made in this laboratory.

Significance to Biomedical Research and to the Program of the Institute: This contractor has pursued a systematic course of research to develop and apply molecular biological methods to demonstrate viral relationships to human tumors. The observations made open new avenues for exploration and development in the study of virally-induced oncogenic processes and their relationship to the spontaneously recurring neoplasms in man.

Proposed Course: The investigations underway will be continued to acquire additional data on the polymerase activities detected in normal embryonic and tumor tissue. The initial studies on the existence of RNA nucleotide sequences in polysomal fractions of human tumor cell which are homologous to the RNA of known animal tumor viruses require further investigations to determine the significance of the observation and expansion of the primary work to other human tumors and virus systems.

Date Contract Initiated: October 29, 1969

Current Annual Level: $800,000

CORNELL UNIVERSITY (NIH-71-2508)

Title: Leukemia Studies in the Cat

Contractor's Project Director: Dr. Charles Rickard

Project Officers (NCI): Dr. Michael A. Chirigos
Dr. Robert A. Manaker

Objectives: To determine mechanisms for transmission of oncogenic viruses in cats and to investigate the possibility of natural infection of humans

by feline viruses.

Major Findings: A suspension type culture of thymic cat tissue has continuously produced FeLV of high particle count per unit volume. Virus was supplied to other investigators and was used for studies on the group-specific antigens of the virion. Antigens have been purified by preparatory electrophoresis and Sephadex chromatography for use in preparing serological reagents. These reagents are considered sufficiently specific for use in sensitive tests to attempt to demonstrate the presence of gs-3 or feline gs-specific antigens in human cancer cells.

FeLV from clones of infected cells was used to produce antisera to fresh undisrupted virus particles. Quantitative assay systems for virus infectivity were compared. Neutralization tests showed that many normal or leukemic cats have serum antibodies neutralizing sub-group A virus. Tests will be conducted to test human sera for antibody against each serotype of the feline C-type viruses.

Some cats with leukemia or related neoplasms have precipitating serum antibody reacting with an unidentified antigen of cat leukemia virus. Sera from some clinically normal cats and some cats in the SPF colony free of conventional cat viruses possess similar precipitating antibody. The antibody appears to be passively transmitted through colostrum. Precipitin-positive sera from normal or SPF cats reacting with this antigen do not give a line of identity with antisera against gs-interspecies or feline gs-species antigens when set up in agar gel tests against disrupted FeLV. The significance of this immunoprecipitin reaction will be sought.

Sera of 8 normal cats which were pets of humans with leukemia were negative in tests for infection by the known feline leukemia viruses. A feline virus was isolated from a cat with lymphosarcoma which was the pet of a human leukemic. In gel diffusion tests against the undisrupted virus isolate, another FeLV serotype, a feline sarcoma virus, and feline group-specific antigens, a five-fold concentrate of the human patient's plasma gave negative results. Sera from 3 members of the patient's household were also negative.

Crandell's continuous feline kidney cell line (CCC) was found useful for assay of FeLV, FeSV, and MSV(FeLV). DEAE-dextran pretreatment enhanced focus formation. The CCC line has shown no evidence of "spontaneous" C-type virus production or gs antigens.

For experimental induction of canine lymphosarcoma by FeLV, age susceptibility is a critical factor. Puppies become refractory within 2-3 days after birth. Time to tumor is substantially decreased by *in utero* inoculation, and virus can be demonstrated in the neoplastic tissue. No virus was detected in puppies born to a female inoculated with FeLV 9 days before delivery suggesting that transplacental infection does not occur.

In other studies it was found that fetal or neonatal dogs inoculated with feline liposarcoma virus developed no neoplasms. Myelocytic leukemia was transmitted from a spontaneous case in a cat to 2 kittens which developed

myelocytic leukemia with abundant C-type particles after 9 months. This virus was inoculated into fetal dogs 16 days before birth. At 5 weeks after birth, there was no evidence of transmission. Fetal inoculation of cats with canine transmissible venereal tumor and canine mast cell tumor was accomplished. Animals are under observation to determine possibility of rescue on oncogenic dog virus genome by the cat.

Significance to Biomedical Research and the Program of the Institute: The cat offers opportunity to determine mechanisms of infection and perpetuation of oncogenic viruses in a natural population. Comparative studies between viruses naturally afflicting different animal species can contribute to an understanding of mechanisms involved in virus-induced cell transformation. The cat viruses infect and reproduce in cells of animal species other than the cat, as well as in human cells. Since the cat is a common household pet, and viral relationships in spontaneous neoplasms have not yet been completely defined, further studies are necessary to determine whether cat viruses may infect humans, particularly children, by contacts in the household.

Proposed Course: Highly specific antisera to the feline viral antigens will be prepared. Serological tests will be applied to experimental and natural cat populations to obtain evidence for possible horizontal transmission of virus infection. Human sera will be tested against the different strains of virus for evidence of human infection. In spontaneous neoplasms of the cat where there is no evidence of the presence of recognized sub-group viruses, efforts will be made to determine whether an unrecognized sub-group is involved. Virus detected or induced in fetal tissues will be compared with the recognized serotypes. Selected canine neoplasms will be studied by different techniques to determine whether the presence of virus may be recognized. Collaborative studies will be conducted with other investigators for evidence of antigenic relationsips between neoplasms of dogs and human patients. The only virus known to produce mammary tumors experimentally is the mouse mammary tumor virus as an important tool for further investigations.

Date Contract Initiated: June 23, 1965

Current Annual Level: $445,500

ALBERT EINSTEIN COLLEGE OF MEDICINE (NIH-71-2251)

Title: Studies on the Molecular Basis of Viral Carcinogenesis

Contractor's Project Director: Dr. J. Thomas August

Project Officers (NCI): Dr. Timothy O'Connor
Dr. Robert A. Manaker

Objectives: To determine the molecular events involved in the adsorption and penetration of oncogenic viruses into host cells, in malignant transformation of cells, and in viral replication.

Major Findings: During the first year tissue culture systems were developed for the study of Friend leukemia virus replication in permissive and non-permissive cells with the aim of determining the mechanism of restriction. Temperature sensitive virus mutants are being selected to help define viral genes responsible for replication and malignancy.

Enzyme studies have shown the presence of a protein kinase within the viruses. This enzyme differs from cellular enzyme and phosphorylates most of the structural proteins of the virion as well as some host cell proteins.

The reverse transcriptase extracted from AMV and RLV, when highly purified, yielded an RNA-DNA hybrid as its sole product. To act catalytically in producing free DNA an AMV endonuclease was required. The transcription of DNA from AMV RNA was increased 10-fold when extracts of AMV were added to the purified enzyme. Fifty percent of the DNA product was single-stranded. Evidence indicates the reverse transcriptase to be a repair type enzyme, and new DNA complementary to the RNA template is covalently bound to the terminal of the DNA primer. Thus, present evidence suggests the reverse transcriptase is not directly involved in the replication of the RNA of tumor viruses.

Significance to Biomedical Research and the Program of the Institute: The studies initiated in the contractor's laboratory are designed to investigate host factors restricting or permitting penetration and replication of oncogenic virus and to probe biochemical events key to viral replicative processes and cellular transformation.

Proposed Course: The virion protein kinase will be purified as a first step to characterization of the enzyme and its biological role in virus infection. The mechanism of cell transformation will be investigated in the Schmidt Ruppin Rous sarcoma virus system. Sensitive quantitative assays of events associated with the transformation process are required. Pathways of carbohydrate metabolism and the time course of appearance of new cell antigens will be explored. To answer questions on the nature of antigenic determinants of the tumor viruses, the first step will be isolation and characterization of the interspecies specific antigen of Rauscher and feline leukemia viruses. Agents which lead to the stimulation of reverse transcription have been partially purified and will be further characterized. Attempts are underway to determine whether the RNA of RLV and AMV contain different or identical 30S subunit components. This information will aid in evaluating the amount of genetic information available for coding in the RNA oncogenic viruses. Studies have commenced on the mechanism of replication of viral RNA. The reverse transcriptase does not appear to be directly involved. Studies are continuing on the synthesis of host cell membrane glycoproteins in infected and uninfected cells. Attempts are being made to purify N and B strains of Friend virus as a preliminary to comparison of their composition. Temperature sensitive Friend virus mutants will be selected and utilized for genetic and biochemical studies directed to elucidation of viral and cellular factors contributing to neoplastic change.

Date Contract Initiated: April 26, 1971

Current Annual Level: $536,000

HAZELTON LABORATORIES, INC. (NIH-69-2079)

Title: Studies on the Etiology of Canine Cancer

Contractor's Project Director: Dr. Erling M. Jensen

Project Officers (NCI): Dr. Michael A. Chirigos
Dr. Robert A. Manaker

Objectives: To determine whether any canine cancer is caused by viruses and whether there is any possible etiological relationship between canine and human cancers.

Major Findings: A common antigen in several specific canine sarcomas was detected by immunofluorescence tests with sera from the tumor-bearing and other sarcomatous dogs. Successful transplantation of a canine mammary carcinoma was achieved by inoculation of beagles in utero. If continuous passage proves possible, the tumor will provide a valuable system for studies on viral etiology, therapy, and possibly hormonal influence on tumor development. Cells of a canine osteosarcoma inoculated into kittens in utero were recovered after birth of the animals and placed in culture. Attempts are underway to determine whether this procedure activated virus production.

A convenient, quantitative infectivity focus assay for feline virus was developed for studies using feline leukemia viruses in attempts to recover defective viruses from canine tumors. Treatment with BUdR and IUdR was introduced in attempts to induce virus release from canine tumor cells. Exposure of XC rat cells, which have been routinely used to titer murine leukemia viruses, to BUdR activated virus production. Two viruses appear to be present.

Studies were initiated to evaluate the effect of combined chemotherapy and nonspecific immune stimulators on spontaneous lymphosarcoma in dogs. In some preliminary tests, streptonigrin was used to determine its inhibitory effect on dog cells infected with feline leukemia virus. Even at toxic drug levels, the reproduction of the virus was not eliminated. Focal areas of morphological change were observed in the canine cells exposed to streptomycin.

Significance to Biomedical Research and the Program of the Institute: Study of canine neoplasms for evidence of viral associations is important in several respects. Since humans are in close contact with canine pets, the possibility that they may be exposed to a canine tumor virus must be investigated. In contrast to the cat and mouse, viruses are not regularly shed by canine tumor cells. An analogous situation exists in the human. The dog provides an excellent experimental animal to determine the presence

of covert viral infections contributing to neoplastic transformations. If such viral relationships to cancer in the dog can be firmly established, the dog will provide opportunity to study the mechanisms for transmission of infection, virus-host relationships, and evaluation of control measures. In this respect, the dog would be one of the best models for human cancer.

Proposed Course: Redirection of the effort in this laboratory is being considered to utilize the facilities for more intensive study on tumor viruses, a more concentrated effort on viral relationships to canine mammary carcinoma, and evaluation of immunotherapeutic approaches to control of naturally occurring neoplasms.

Date Contract Initiated: May 26, 1969

Current Annual Level: $400,000

JOHNS HOPKINS UNIVERSITY (NIH-71-2121)

Title: Herpesvirus Antigens and Virions in Neoplastic Cells from Squamous Carcinoma of the Human Cervix

Contractor's Project Director: Dr. L. Aurelian

Project Officers (NCI): Dr. Charles W. Boone
Dr. Robert A. Manaker

Objectives: The ultimate objective of this project is the development of evidence for/or against virus as a factor in the etiology of carcinoma of the human uterine cervix. The immediate objective is the identification of Herpes simplex virus type II (HSV-2) antigens and virions in neoplastic cells.

Major Findings: Over 90% of patients with epithelial atypia, carcinoma in situ, or invasive carcinoma of the cervix possessed antibodies to HSV-2 in contrast to positive findings in only 55% of a matched control population. HSV-2 antigens were detected in the cytoplasm of exfoliated dyskaryotic cells of cancer patients by immunofluorescence. In tumor biopsies, no evidence of virus particles or antigens were detected. Cultured cells from a cervical carcinoma could be induced to release virus by raising the pH of the culture medium.

Significance to Biomedical Research and the Program of the Institute: An important question relevant to total program is whether Herpes viruses are directly responsible for oncogenesis or whether infections activate a pre-existing premalignant state. The Special Virus Cancer Program is concentrating on studies of neoplastic diseases in man and animals in which Herpes virus involvement has been demonstrated in order to define a specific role, if any, for this group of viruses in the neoplastic process. If genital Herpes virus infection can be definitely shown to be a factor in the development of cervical neoplasia, appropriate control measures may be developed to reduce the incidence of this cancer in man.

Proposed Course: The contractor will compare antigens in cervical carcinoma cells with those induced in Hep-2 by Herpes simplex virus type I and II. Work will continue on the characterization of HSV-2 antigens present in exfoliated cancer cells from patients with cervical cancer.

Date Contract Initiated: May 5, 1971

Current Annual Level: $92,000

KAROLINSKA INSTITUTE (NIH-69-2005)

Title: Studies on the Significance of Herpes-type Virus in the Etiology of Some Human Cancers

Contractor's Project Director: Dr. George Klein

Project Officers (NCI): Dr. Charles W. Boone
Dr. Gary R. Pearson

Objectives: (1) To obtain additional data on EB virus-host interactions. (2) To investigate host immune responses to tumor antigens. (3) To study the regulation of C-type virus expression in defined systems. (4) To investigate cell mediated tumor immune reaction mechanisms in vitro and in vivo.

Major Findings: EBV related research: membrane antigens, early antigens and virus capsid antigens mediated by EBV have been studied in established lymphoblastoid cell lines. Inhibitors such as mitomycin C increase the amount of antigens detected by immunofluorescence. Mouse lymphoblastoid cell hybrids have made it possible to determine whether the presence of EBV DNA is dependent on one or several chromosomes. BUDR labeled cells were super-infected with tritiated thymidine labeled virus and the heavy cell DNA was recovered and examined for associated EBV. No evidence of integrated EBV genome was found. The incubation of tumor cells with serum from patients with lipo-, fibro-, osteo-, and neuro-sarcoma inhibited the stimulation in one autologous lymphocyte-fibrosarcoma combination.

Significance to Biomedical Research and the Program of the Institute: A major effort of the Special Virus Cancer Program has been the study of the viral involvement in the etiology and course of Burkitt's tumor in man. Research on the relationship between EBV infection and the onset of Burkitt tumor, the development of EBV-coded antigens in infected cells, and the analysis of the immune response to Burkitt tumor is therefore highly relevant to total program.

Proposed Course: The contract effort will continue essentially as described above.

Date Contract Initiated: April 9, 1968

Current Annual Level: $90,000

LIFE SCIENCES, INC. (PH43-69-63)

Title: Studies on Marek's Disease as a Model for Herpesvirus Associated Oncogenesis

Contractor's Project Director: Dr. Jack Frankel

Project Officer (NCI): Dr. Gary Pearson

Objectives: The objectives of this project are (1) to determine the exact nature of the role of the herpesvirus associated with Marek's disease in the etiology of this disease, i.e. whether it is direct, or indirect (e.g. by interacting with some other agent, or by activating a covert viral oncogene-the Huebner hypothesis); (2) to establish Marek's disease as a model system for herpesvirus associated oncogenesis, using reagent grade SPF avian hosts (free of overt leukosis virus and other pathogens); and (3) to operate an avian virus testing laboratory for monitoring both hosts and viral materials used in experiments for freedom from extraneous viruses, including infectious leukosis virus.

Major Findings: In vitro interference studies were conducted with new standard pools of an avian leukosis virus (RAV-2) and cell free Marek's disease herpesvirus (MDHV). The results showed that a marked reduction in MDHV foci occurred in primary and secondary cultures of LSI-SPF CEF cells infected with RAV-2 and superinfected with MDHV. Preliminary interference studies also were performed in conventional chickens. When embryonated eggs were inoculated with RAV-2 and the chicks superinfected with MDHV by inoculation or contact with infected chicks, a marked decrease in MDHV neutralizing antibody titers occurred. In another interference test performed in vivo, deaths of LSI-SPF chickens infected with both Rous sarcoma virus (Bryan) (RSV) and MDHV were delayed three to five days as compared to chickens infected with MDHV alone. The latent period to development of the RSV-induced wing tumors was decreased by one day in groups inoculated with both viruses. Following infection of conventional chicks with MDHV, infectious MDHV could be extracted from feather follicle epithelium 11 days after infection. Viral antigen was demonstrated in feather follicle epithelium 13 days later and persisted for at least 56 days; antibodies were not detected until 49 days had elapsed. These results were in agreement with data from previous studies in which MD tumor cell suspension were used as inocula. Inoculation of LSI-SPF chicks with the GA isolate of MDHV obtained from another source, and with a passage history differing from that of the GA isolate of MDHV used in these laboratories, resulted in the same pattern of early mortality. The studies were performed in MDHV contaminated and isolator environments both with cell-free and cell-associated virus. In marked contrast to the results, no mortality occurred amongst LSI-SPF chicks housed in an isolator after inoculation with the cell associated HPRS-16 isolate of MDHV. However, MDHV was recovered from extracts of skin and feather follicle epithelium from these chickens five weeks after inoculation.

Passive immunization of LSI-SPF chicks with MDHV antiserum resulted in a marked decrease in the characteristic mortality rate induced by inoculation with MDHV. These chickens were housed in MDHV contaminated environment. On

the other hand, administration of the same antiserum and MDHV challenge to LSI-SPF chicks housed in germfree isolators did not result in any evidence of a protective effect.

Preliminary studies relating to the pathogenesis of Marek's disease in LSI-SPF chicks housed in isolators and infected with cell-free MDHV was studied by sequential changes in histopathology. The results showed a marked progressive hyperplasia of the reticuloendothelial tissues which was seen most prominently in the major lymphoid organs (thymus, bursa of Fabricius and spleen). In the terminal stages of the disease, the lymphoid tissues were completely replaced by reticular cells to the exclusion of recognizable lymphocytes. There was also a complete replacement of adult lymphocytes by reticular cells in all organs where perivascular foci of lymphocytes are usually seen.

Significance to Biomedical Research and the Program of the Institute: No satisfactory animal model is currently available for studying the exact role (i.e. whether direct or indirect) of herpes-type viruses in the induction of neoplasia. Since herpes-type viruses are associated in very high frequency with two types of human cancer, namely Burkitt's lymphoma and post-nasal carcinoma, an animal model system for developing approaches to and guiding studies on the human problem is urgently needed.

Since Marek's disease of chickens is also associated with a herpesvirus (MDHV), and this virus can now be isolated and worked with systematically in the laboratory, it seems likely that this chicken disease can be developed into the desired laboratory model.

Proposed Course: Investigations on interference and enhancement interactions between various MDHV isolates and other viruses, such as avian leukosis and other adventitious agents, both in vitro and in vivo, will be continued. The relative importance of cell mediated and humoral immunity will be investigated in experiments designed to closely follow the development and regression of Marek's disease in LSI-SPF and conventional birds.

The nature of the different responses of LSI-SPF chickens to inoculation with a variety of MDHV isolates will be investigated in terms of virology, immunology and pathology. A comprehensive study to determine the inter-relationships between virus source, dose, host, age, route of inoculation and type of chicken as related to virulence and immunocompetence will be performed.

Date Contract Initiated: November 1, 1968

Current Annual Level: $458,600

MASSACHUSETTS GENERAL HOSPITAL (NIH-71-2174)

Title: Characterization of Nucleic Acids of the Avian Myeloblastosis Virus

Contractor's Project Director: Dr. Paul C. Zamecnik

Project Officers (NCI): Dr. Timothy E. O'Connor
Dr. Robert A. Manaker

Objectives: To analyze the end groups and acromolecular sequencing of the large molecular weight RNA of avian myeloblastosis virus (AMV) and to analyze the minor base composition of the transfer RNA of AMV.

Major Findings: High molecular weight RNA was prepared from AMV to determine the nucleotide sequence of the molecule adjacent to the terminal ends. At present, only the 3'OH terminal nucleoside has been determined. Adenosine was found to be the predominant terminal residue, followed by cytidine. Uridine comprises 15% or less of the terminal residues in these experiments. The main impediment to sequencing this RNA has been the highly heterogenous character of the material as isolated. The purest preparations of 35 S material to date contain at the 3'OH end 67% adenosine and 15% cytidine. Since avian tumor viruses as well as other oncorna viruses are known to be mixtures of closely related viruses, end group studies may indicate the predominant type of virus present in a mixture, although related viral species may also have the same terminal sequence. These results are not in agreement with others who have reported the 3'OH end to be uridine.

A comparison was made of the base composition of tRNA from AMV with that from Cofal-negative chick liver and from infected myeloblast tRNA. Small but significant differences in content of major bases are present between virus and host cell tRNA. More striking differences exist in certain minor bases. In virus tRNA, lower levels of all modified pyrimidines (except pseudouridine) and levels of all methylated guanines are higher than in the host cell tRNA. AMV tRNA has all minor bases so far found in host tRNA and no evidence exists of additional bases in AMV tRNA. However, a number of minor bases have not yet been assayed by this method. The content of 3-methylcytosine in the virus is 1/5 the level in the cell. This is a rare minor base present in but 12-25% of individual host cell tRNA molecules.

Recent findings suggest that the "70 S-associated" 4 S RNA contains in addition to the 4 major bases, the minor bases: pseudouridine, dihydrouridine, and 5-methylcytosine. Other minor bases may be present. At this time it is reasonable to infer that this 4 S RNA can qualify as tRNA. Preliminary work showed adenosine to be the predominant terminal nucleoside.

Fifteen primary and secondary cultures of spleen cells from Hodgkin's patients were pulsed with ^{3}H-uridine and five cultures with ^{3}H-thymidine. Upon centrifugation of culture fluids, radioactivity occurred in the 1.16 region. Pelletized fractions from the 1.16 region were examined by electron microscopy and particles suggestive of C-type were seen.

Significance to Biomedical Research and to the Program of the Institute:

The elucidation of the nucleotide sequences of the 70 S RNA of AMV and other oncogenic RNA viruses may reveal segment active in specific functions and as binding sites of viral polymerases or inhibitors thereby increasing our knowledge of transcription processes. Elucidation of the differences in tRNA-encapsulated in virions and those present in normal and virus-infected cells may show how virus infection dominates translation as well as transcription processes. Hodgkin's disease has been suspected to be virally-induced. Recent observations suggest this may be true. An excellent opportunity exists in this laboratory to investigate viral relationships in this disease.

Proposed Course: The analysis of nucleotide sequences at terminal ends of large molecular weight tumor virus RNA will continue. A more detailed analysis of the minor bases of AMV tRNA will be conducted. The minor base constitution of the 70 S-associated 4 S RNA of AMV will be scrutinized. Pilot studies on viral associations with Hodgkin's disease will continue.

Date Contract Initiated: June 29, 1971

Current Annual Level: $95,790

MASSACHUSETTS GENERAL HOSPITAL (NIH-72-2012)

Title: Activation of Oncogenic Viruses and Induction of Cancer by Immunologic and Non-immunologic Methods

Contractor's Project Director: Dr. Paul Black

Project Officers (NCI): Dr. Adi Gazdar
Dr. Michael Chirigos

Objectives: To determine the relationships between chronic allogeneic disease, immunosuppression, and interferon inducers on the activation of covert infections by oncogenic RNA viruses.

Major Findings: The graft versus host reaction (GVHR) in vivo and its in vitro counterpart the mixed lymphocyte culture (MLC) were used in this study. Test systems were assayed for virus by the Klement XC and Bassin S+L-techniques. The test systems included: (1) Spleen cells from ($C_{57}Bl_6$ x DBA) F_1 mice (hereafter called BDF_1), either assayed directly, or cultured for 3 days in vitro. (2) Spleen cells from BDF_1 mice undergoing a GVHR (previously inoculated with DBA/2 cells), directly or cultured for 3 days in vitro. (3) Spleen cells from (BALB/c x A/J) F_1 mice (hereafter called CAF_1), as above. (4) Spleen cells from CAF_1 mice undergoing a GVHR (previously inoculated with BALB/c spleen cells, with or without mitomycin-C. (6) Spleen

cells from CAF_1, BALB/c, A/J mice cultured separately with or without phytohemagglutinin (PHS), concanavalin-A (con-A), or iododeoxyuridine (IUDR).

Cultures were tested for proliferation by the addition of 1 μCi-^{3}H-thymidine 4 hours before termination of cultures and assessed by standard liquid scintillation techniques. All cultures were coded and assayed for leukemia viruses blindly.

Activation of leukemia virus during the GVHR. Cultures of spleen cells from uninoculated CAF_1 and BALB/c mice were negative for leukemia viruses by the XC or S+L- assays, or both in 15 of 16 experiments. However, cultures of spleen cells from CAF_1 mice previously inoculated with parental BALB/c cells were positive for leukemia virus by the same assays in 10/11 experiments; titers in these cultures ranged from $10^{1.2}$ to greater than $10^{3.2}$ $TCID_{50}$ (50% tissue culture infectious doses)/ml.

In contrast to the results observed with CAF_1 cells, leukemia viruses were infrequently recoverable from BDF_1 spleen cell preparations, and in 4 of 16 allogeneic spleen cell preparations. Titers in the positive BDF_1 cultures did not exceed $10^{1.2}$ TCID 50/ml.

Activation of leukemia viruses in mixed lymphocyte cultures (MLC). Three to seven day old cultures of mixtures of BALB/c and CAF_1 spleen cells were positive for leukemia viruses by the XC or S+L- assays, or both, in 7 of 11 experiments. In three cultures which did not produce virus, lymphocyte stimulation was sub-optimal, with isotope incorporation ratios of only 2.5 x control levels, whereas the 7 positive cultures were stimulated at a minimum of 5x the control level. CAF_1 and BALB/c spleen cells cultured separately for the same periods were negative for viruses.

Relationship of phytohemagglutinin-induced blastogenesis to virus activation. Lymphocytes of CAF_1, BALB/c, or A/J mice cultured in the presence of PHS underwent marked blastogenesis but did not release virus following this treatment. Similarly, titers of leukemia virus from cultured spleen cells of CAF_1 animals undergoing a GVHR were not altered by PHA treatment ($10^{1.2}$ $TCID_{50}$ per ml with or without PHS). The proliferative response of spleen cells from CAF_1 mice with the GVHR to PHS was significantly less than that of normal CAF_1 spleen cells. Spontaneous blastogenesis in these cultures during the 1st day was 2.8 x control values, but fell below controls by day 3.

Both leukemia virus positive MLC's and leukemia virus negative PHA cultures showed proliferative responses as measured by isotope incorporation. Mitomycin-C treatment of responding BALB/c cells prevented both blastogenesis and virus release. Both BALB/c and CAF_1 lymphocytes when cultured with IUDR released leukemia viruses in preliminary trials; in addition IUDR increased the yields from MLCs.

These results would appear to be the first instance of in vitro activation of leukemia viruses by immunological techniques. Activation does not appear to be solely the result of transformation of lymphocytes because PHA induced marked blast transformation in CAF_1 lymphocytes, yet activation did not occur. Furthermore, PHS did not increase the titer of recoverable leukemia virus in lymphocytes from mice undergoing GVHR. Similarly, in vivo challenge of CAF_1 mice with sheep erythrocytes, allogeneic cells, and Freund's adjuvant did not activate leukemia viruses; studies using in vitro antigenic challenge of lymphocytes from immunized animals are in progress. A non-immunological mechanism that could activate leukemia viruses seems remote because of the failure to find virus release upon co-cultivation of CAF_1 and mitomycin-treated BALB/c spleen cells.

The biological significance of the leukemia virus activation is apparent since treatments which prevented cellular proliferation and virus activation, e.g., mitomycin-C treatment of donor cells, were also shown to prevent the GVHR and lymphoma development. In addition, none of 81 BDF_1 mice developed a lymphoma following the injection of DBA/2 spleen cells and in this combination leukemia virus was infrequently detectable in vitro or in vivo.

Activation or induction of murine leukemia virus from normal embryo cells.

Primary embryo cultures were established in routine fashion from mice of the following strains: Charles River Swiss CD[1], spf C57Bl/6 MI. Various procedures were attempted to induce MuLV from these cells. The presence of virus was determined by the XC test. Virus was recovered after 2 weeks of leucine deprivation; a questionable positive XC test was obtained with the 5-week leucine deprivation samples. Attempts to induce MuLV from embryo cells following treatment with mitomycin-C have not been successful.

Significance to Biomedical Research and the Program of the Institute:

This contract contributes important new information about the relationship between immunologic abnormalities and enhanced susceptibility to oncogenesis of suspected viral origin. A better understanding between the two may be of value in the formulation of approaches to the prevention of malignancies developing in the wide range of human disease states associated with immunologic abnormalities.

Proposed Course: The studies completed strongly suggest that lymphocyte-lymphocyte interactions can activate leukemia viruses in vivo and in vitro. The relationship to lymphomas occurring in patients with autoimmune disease or transplantation reactions is obvious. The contractor will attempt to determine whether virus is activated in the donor's or the recipient's cells by labeling techniques and electron microscope localization. Studies will be continued to determine whether other treatments activate covert virus infections. Lymphocyte-fibroblast

and fibroblast-fibroblast interactions will be followed for evidence of viral activation, and further work will be carried out on the induction of mouse leukemia virus from normal embryo cells.

Date Contract Initiated: September 15, 1971

Current Annual Level: $74,370

MERCK AND COMPANY, INC. (NIH-71-2059)

Title: Oncogenic Virus Research and Vaccine Development

Contractor's Project Director: Dr. Maurice Hilleman

Project Officers (NCI): Dr. Robert A. Manaker
Mr. J. Thomas Lewin

Objectives: To conduct investigations designed to develop vaccines or other agents effective for the prophylaxis and therapy for human neoplasia of suspected viral etiology.

Major Findings: Multiple construction and renovation projects have been involved in the expansion and reorientation for this program. Remodeling of a laboratory, physically separated from the animal tumor virus area, was recently completed and is in use for Herpes simplex type 2 vaccine work. Two rooms (440 sq. ft.) in Bldg. #43 were remodeled and equipped and are in use for the germ-free derivation of kittens for the SPF cat colony breeding nucleus. Plans were completed for the renovation of half of Bldg. #65 (5,940 sq. ft.) for housing an SPF cat colony and for housing experimental cats. The construction and equipping of the new biohazard containment building #26B (12,096 sq. ft.) for laboratory work is progressing on schedule. The projected completion date is September, 1972.

Tumor-specific cellular vaccine development: The preparation and assay of tumor cell vaccines for protective efficacy in the hamster model system was continued at a lower priority level. Testing of adenovirus 31 tumor cell fractions prepared by mechanical disruption of the cells and fractionation by differential centrifugation was completed. None of the vaccines (crude cell homogenate, nuclear fraction-$\omega^2t = 10^7$ pellet, membrane fraction-$\omega^2t = 5 \times 10^9$ pellet, particulate fraction-$\omega^2t = 10^{11}$ pellet, cell sap-$\omega^2t = 10^{11}$ supernate) protected hamsters against development of tumors when they were challenged by inoculation of viable homologous tumor cells. Work on the preparation of two other types of tumor cell antigens was continued. Cell membranes were prepared from a adenovirus 12 tumor cells by hypotonic extraction and were solubilized by sonication. The solubilized material was fractionated on Sephadex G200

columns and the desired fraction concentrated by the Diaflo membrane technique. The first batch of test and control antigens is on test for protective efficacy in hamsters. Preparation of additional batches of antigen for assay is in progress. Technology is still being developed for the preparation of adenovirus 7 tumor cell membranes by flow sonication and flow zonal centrifugation.

Investigation of the host immunologic response to nonprotective tumor cell vaccines is being conducted in hamster-tumor model systems. The first series of experiments was designed to test the effect of inoculation of known nonprotective vaccines before, simultaneously with, or after immunization with a known effective vaccine (5×10^6 γ-irradiated tumor cells). Most of the experiments in this series are on test. Final results with one of the nonprotective vaccines, SV_{40} tumor cell ghosts prepared by hypertonic extraction, showed that this vaccine did not interfere with the ability of the host to reject viable homologous tumor cells after vaccination with 5×10^6 γ-irradiated SV_{40} tumor cells.

Attempts to render nonprotective SV_{40} tumor cell vaccines effective by the administration of poly I:C before, simultaneously with, or after vaccine, single or multiple doses, or by different routes were not successful in the hamster model system.

Studies on the role of fetal antigens in tumor immunology are being conducted in the SV_{40}-hamster model system. In the first series of experiment, γ-irradiated, 9-12 day gestation fetal cells of multiparous origin did not protect adult male or female hamsters against tumor development when challenged with 5000 homologous tumor cells. Experiments are in progress wherein the vaccines were prepared from primaparous 10-day gestation embryos and are being tested in the SV_{40} virus-newborn hamster model system and in the adult hamster-tumor cell challenge system with a 2500 cell challenge dose.

Virus vaccine development: This project is still in the initial stages. The work in progress is concerned primarily with basic needs such as virus propagation, virus concentration and purification, preparation of specific antisera, and establishment of routine assay procedures.

The KT (Kawakami-Theilen) strain of feline leukemia virus (FLV) was routinely propagated in roller bottle (1 liter/bottle) suspension cultures of the virus-shedding FL74c cell line. Ten liter lots of culture fluid were concentrated (1000x) and purified by flow zonal centrifugation and isopynic centrifugation on sucrose gradients. Modifications in technology are still being made to increase the purity of the concentrated virus. Virus yields of 10^{13} virus particles/ml were readily achieved.

In order to provide an adequate supply of healthy cats for future experimental work, establishment of a specific pathogen-free cat colony was proposed. The first step, the germ-free derivation of the breeding

has been in progress for two months. All eight isolators are occupied by kittens (16 females, 7 males) ranging from 1 to 8 weeks in age.

Significance to Biomedical Research and the Program of the Institute:

If viruses are an essential element in the genesis of some human cancers, prophylaxis by vaccines to prevent or minimize infection should provide a rational approach to cancer prevention. This could be accomplished by living or killed virus vaccines or possibly by vaccines of purified virion sub-units. Although greatest benefit could be derived by prevention of infections transmitted horizontally after birth, a potential benefit from vaccines may be derived where viruses are transmitted vertically but do not express their full antigenic complement. Non-oncogenic viruses may function as essential co-factors in expression of neoplasia, and immunity against such secondary agents might prevent expression of the neoplastic state. In addition, vaccination with homologous virus in a virus-dependent cancer may enhance specific humoral antibody or cellular immunity. This research project is of fundamental importance to total program.

Proposed Course: Efforts to prepare tumor-specific cellular antigens for immunoprophylaxis of cancer and to study the immunologic response to such antigens will continue. Tests with poly I:C for adjuvant effect on ineffective cellular vaccines will be completed. Work towards development of a feline leukemia-sarcoma virus vaccine and a herpesvirus type 2 vaccine will be continued as rapidly as possible. If no problems arise, the germfree derivation of kittens for the SPF cat colony should be completed in several months.

Date Contract Initiated: March 1, 1971

Current Annual Level: $1,016,000

UNIVERSITY OF NAPLES (NIH-71-2056)

Title: Studies of Non-Virion Antigens

Contractor's Project Director: Dr. Giulio Tarro

Project Officer (NCI): Dr. Charles W. Boone

Objectives: To determine whether Herpes simplex virus-specific non-virion antigens occur in certain types of human tumors as evidence of the incorporation of viral genome in tumor cells. To search in human sera obtained from selected cancer patients for the presence of antibodies which react specifically with herpesvirus non-virion antigens.

Major Findings: To determine whether herpesvirus non-virion antigen produced early post-infection with the Schooler strain HSV-1 was strain

specific, six strains recovered from oral lesions were used to produce non-virion antigen. No non-virion antigen reactive with antibodies to the Schooler strain antigen was detected with 4 of the strains while 2 reacted positively. This antibody also did not react with a type 2 genital herpes strain. In collaboration with Dr. Ariel Hollinshead the antiserum to HSV-1 non-virion antigens was found to react with soluble cell membrane antigens extracted from lip carcinoma and from cervical carcinoma.

The contractor has shown recently that the 4 strains of HSV-1 do produce non-virion antigens because antibodies induced in guinea pigs hyper-immunized with these strains react with Schooler strain HSV-1 reference antigens. HSV-1 strains probably induce multiple antigens of the non-virion variety in different concentrations accounting for the variability in reactivity in specific systems. Antibodies have been prepared against HSV-2 non-virion antigens. These react both with HSV-2 antigens and antigens from most strains of HSV-1. Apparently the component of HSV-2 antigens engendering antibody to HSV-1 antigen is present in insufficient amount in infected cells to be detected by the antiserum to the Schooler strain HSV-1 antigens. Therefore, to establish the role of herpes-viruses in certain human tumors, many sera from patients with these tumors must be tested against these non-virion antigens, or antisera to these non-virion antigens must be tested against antigens of known potency from selected tumors, because these antigens, while capable of stimulating antibodies, may be below the limits for detection by the procedures used.

Significance to Biomedical Research and the Program of the Institute:

Certain herpesviruses have been etiologically associated with lympho-proliferative diseases in man and animals. Two other members of the herpesvirus group have been suspected to be of etiological significance in the development of the renal carcinoma of the frog and cervical carcinoma in humans. This particular contract was instituted to develop serological reagents for detecting non-virion antigens expressed early after infection of cells by herpesvirus. Such reagents would be useful for evaluating the relationship of herpesviruses to oncogenic processes in man.

Proposed Course: The contractor will examine certain types of human tumors for evidence of the presence of non-virion herpesvirus antigens and search human sera from cancer patients for antibodies that react specifically with the herpesvirus non-virion antigens.

Date Contract Initiated: April 9, 1971

Current Annual Level: $30,000

OHIO STATE UNIVERSITY (NIH-69-2233)

Title: Application of the Radioiodine Labeled Antibody Technique to Studies on Virus-induced Tumors

Contractor's Project Director: Dr. David S. Yohn

Project Officers (NCI): Dr. Jack Gruber
Dr. Virginia C. Dunkel

Objectives: To apply the paired radioiodine labeled antibody technique (PRILAT) to the detection of virus-induced tumor antigens in human tumor cells.

Major Findings: The paired radioiodine labeled antibody technique (PRILAT) has been adapted for use in serologic studies of human sera for antibodies to mammalian oncornavirus antigens. The direct PRILAT inhibition test has been used. Antibody (IgG) to ether-extracted FeLV or MSV has been labeled with ^{125}I, and normal IgG of the same animal species has been labeled with ^{131}I and mixed in equal protein concentrations. The test consisted of attempting to block the reaction of anti-FeLV or anti-MSV with formalin-acetone fixed cells known to contain oncornavirus antigens. The same sera were tested by direct complement-fixation (CF) and by CF inhibition tests using soluble antigens obtained from oncornavirus producing cell cultures. The latter tests have been used to test for antibodies to the gs-3 mammalian interspecies oncornavirus antigen. Tests conducted on over four hundred sera obtained through the SVCP have provided presumptive evidence of antibodies to oncornavirus antigens in human sera, particularly those from patients with breast carcinoma, lymphosarcoma, liposarcoma, Hodgkin's disease, rhabdomyosarcoma, reticulum cell sarcoma and acute lymphocytic leukemia.

Significance to Biomedical Research and the Program of the Institute:

This project was designed to apply PRILAT as a very sensitive tool for the detection of specific reactions between humoral antibodies and tumor cell surface antigens. Because the method permits discrimination between specific and non-specific binding of serum globulins, it is a valuable tool in the study of human tumor antigens.

Proposed Course: This contract terminates on June 26, 1972. Important aspects of the work will be incorporated into another contract.

Date Contract Initiated: June 27, 1969

Current Annual Level: $90,000

OREGON STATE UNIVERSITY (NIH-71-2175)

Title: Studies on the Replication and Function of Nucleic Acids Isolated from Oncogenic Viruses

Contractor's Project Director: Dr. George S. Beaudreau

Project Officer (NCI): Dr. Albert J. Dalton
Dr. Ursula Heine

Objectives: To study the enzymatic and biochemical changes occurring during cellular transformation by oncogenic virus and to relate these biochemical modifications to observable ultrastructural events and alterations.

Major Findings: Chick embryo cells infected with MC29 tumor virus release virions into the culture medium within 10 hours after infection. In this short period the cells must establish the necessary biochemical reactions for virus replication, and it seems that some of the heretofore inaccessible aspects of early virus infection might be examined. The contractor has chosen initially to look at the fate of the nucleic acid in the infecting virion and to look for and follow the development of the virus-specific DNA polymerase in the cell infected with tumor virus.

There is found in the virus-infected cells a DNA polymerase activity that is many-fold stimulated over that found in control cells. This enzyme has a sedimentation coefficient that appears to be slightly greater than 8 S. This may be larger than the sedimentation coefficient of the DNA polymerase as isolated from the virus (7.6 - 7.8). The enzyme appears to be associated with the outer membrane. There is little evidence for this enzyme increasing in the cell prior to release of virus. Indeed, the large accumulations are detectable 48 hours after infection.

The contractor has studied the fate of the MC29 virus RNA within the infected cell during the eclipse period of replication, when no free virus can be detected. MC29 virus, labeled with ^{3}H-uridine, was adsorbed to the cells and after short periods the cell homogenates were examined for ^{3}H-labeled virus RNA. Initial experiments have shown virus RNA in association with the polysome fraction of the infected cells.

Significance to Biomedical Research and to the Program of the Institute:

This project was undertaken as a collaborative study with investigators at NCI to determine the intracellular sites at which specific events in the very early stages of virus infection and replication occur. Such information obtained on a defined virus-host cell system under controlled conditions is important for the interpretation and significance of data acquired on other malignancies.

Proposed Course: The present approach is under evaluation. The continuation of this project will depend upon the assessment of the data acquired and possible refinements in technique.

Date Contract Initiated: June 28, 1971

Current Annual Level: $33,640

PENNSYLVANIA STATE UNIVERSITY (NIH-70-2024)

Title: Studies on the Oncogenic Potential of Defective Human Viruses

Contractor's Project Director: Dr. Fred Rapp

Project Officers (NCI): Dr. Robert A. Manaker
Dr. Jack Gruber

Objectives: To conduct a systematic study of the oncogenic potential of defective human viruses.

Major Findings: Additional cell lines were developed following exposure to HSV-2 previously inactivated with ultraviolet light. These cells were shown to be oncogenic when inoculated into newborn hamsters. A number of these cell lines have been characterized and shown to contain herpes-specific antigens in a proportion of the cells in culture. These antigens cause the production of antibodies in tumor-bearing animals that react with cytoplasmic antigens and are capable of neutralizing herpes simplex virus type 2. Thus far, no infectious viruses have been recovered from any of the tumor cell lines. In addition, no C-type virus particles have been observed nor have tests for antigens specific for such virus been detected in preliminary experiments carried out in this laboratory.

The tumors have a tendency to metastasize to the lung, liver, and kidney. The histopathology of the tumors suggests interlacing bundles of pleomorphic fibroblasts with tumors composed of poorly differentiated anaplastic sarcomatous tissue. The tumors infiltrated to the surrounding musculature but did not invade bone.

In further studies, thirteen herpes simplex virus (HSV) type 1 and type 2 isolates were tested for transforming ability of hamster embryo fibroblasts (HEF) following exposure of the viruses to ultraviolet light. Two of the new HSV-2 isolates have induced transformation of hamster cells and isolation and characterization of these cells is now in progress. Preliminary results suggest that they have similar properties to the previously described transformed cells. Attempts to detect C-type particles have been negative in 8 cell lines examined thus far.

Hamster cells transformed by chemically inactivated HSV-2 have now produced tumors when inoculated into neonatal Syrian hamsters. Cells derived from those tumors maintain the properties of the parental lines concerning resistance to infection by HSV-2 while supporting the replication of HSV-1.

Attempts to activate HSV in human cells in which the virus is latent using a variety of agents failed. Radioisotope experiments revealed that the latently infected cells contained parental virus DNA with about 5% of the input radioactivity recoverable. In addition, similar latent infection of cells from patients with xeroderma pigmentosum yielded extended latent periods in the presence of ara-C before virus began to be resynthesized after the inhibitor was removed. The overall results suggest that repair of the virus genome may be necessary as a prelude to renewed synthesis of infectious virus.

Host dependent restrictions on HSV replication appeared to be especially pronounced at 39° C. Comparison of infectious virus yields at that temperature revealed that all HSV-1 strains tested replicated in hamster cells at the higher as well as at lower temperatures. In contrast, HSV-2 strains did not replicate at 39° C in hamster cells although the virus was able to replicate in rabbit kidney cells at that temperature. These results suggest fundamental difference in the replication of HSV-1 and HSV-2.

Further experiments with EBV have yielded results that suggest that this virus is able to interact with HSV-2 in hamster cell lines non-permissive for either virus. Doubly infected cells produced HSV-2 antigens and synthesized infectious virus more effectively than cells infected only with HSV-2 (where replication was very poor). Neither EBV antigens nor infectious EBV was detected in the system. The role of EBV in enhancing the replication of HSV-2 is being further studied.

Significance to Biomedical Research and the Program of the Institute:

The neoplastic response to oncogenic virus infection is a slow process in contrast to common virally-induced diseases. This project was established to determine whether common viruses, which are defective in their capacity to induce a cytolytic cycle of reproduction, may establish a chronic infection terminating in neoplasia. This is an important aspect of Program. Current observations have shown that one virus, having been rendered defective, does induce oncogenesis in animal cells. This virus, a genital strain of herpesvirus, is particularly important, since it has been suspected to be related to the development of cervical carcinoma in humans.

Proposed Course: The present studies will be continued to further investigate in animals and human cells the relationships of defective herpesvirus type 2 to the oncogenic process observed. Emphasis will

also be given to EB virus and cytomegalovirus and to interactions between the herpes viruses.

Date Contract Initiated: October 27, 1969

Current Annual Level: $448,000

UNIVERSITY OF PENNSYLVANIA (PH43-65-1013)

Title: Research On Experimental and Natural Transmission of Bovine Leukemia

Contractor's Project Director: Dr. Robert Marshak

Project Officers (NCI): Dr. Michael A. Chirigos
Dr. Robert A. Manaker

Objectives: To investigate the possible viral etiology of bovine leukemia, attempt cell-free transmission of bovine leukemia by cell-free preparations, and investigate natural transmission of the disease through reciprocal foster nursing experiments.

Major Findings: A number of cell lines prepared from leukemic cattle have been examined for the presence of virus. Cell line NBC-13 was incubated with uridine-^{3}H. The culture fluid and a cell homogenate were subjected to low speed centrifugation followed by high speed sedimentation of supernatants. The pellets were subjected to centrifugation in a density gradient. The gradient fractions in which a peak of radioactivity was detected were found to contain particulates similar to virus of the C-type. The presence of the uridine label suggests that these particulates contain RNA. Production of these particulates in available cultures has been erratic, and sufficient material for more intensive study has not been acquired.

Gamma globulin separated from the serum of an animal in which regression of bovine leukemia occurred reacted in immunofluorescence tests with antigens in cells of the bovine leukemia cell line. Tests strongly indicate that these antigens are not the result of murine or feline virus contamination. Immunodiffusion tests also failed to detect the presence of the gs-3 interspecies antigen using antisera prepared against internal virion antigens of murine and feline leukemia viruses.

Significance to Biomedical Research and the Program of the Institute:

Epidemiological surveys suggested that bovine leukemia might be induced by a viral factor. Investigation was important to establish whether the observations of virally induced leukemia in avian and murine species was indicative of similar relationships in higher species, whether

horizontal spread of such tumor virus infections was a factor in the occurrence of disease in an animal population, whether there were close antigenic or genetic relationships between the viruses of different species, and whether bovine viruses might pose a hazard to humans. The observations made suggest that virus associations with bovine leukemia do exist. Further study is required to determine the significance of the association.

Proposed Course: Studies will continue to attempt to increase virus production in bovine leukemia cell lines to permit further characterization of the virus particulates observed. A reduction in the annual budget is under consideration.

Date Contract Initiated: June 18, 1965

Current Contract Level: $300,000 for 6 months

THE PUBLIC HEALTH RESEARCH INSTITUTE OF THE CITY OF NEW YORK, INC. (NIH-71-2129)

Title: Evaluation of Methods for Isolation of Virus from Human Neoplasia

Contractor's Project Director: Dr. Hidesaburo Hanafusa

Project Officers (NCI): Dr. Charles W. Boone
Dr. Robert A. Manaker

Objectives: (1) To apply the method successfully used for isolating oncogenic viruses from covert infections in animals to the recovery of viruses from human cancers. (2) To characterize viruses recovered from human cancers.

Major Findings: Nineteen fresh human sarcoma specimens were studied. Tissue cultures of these tumor cells were examined for the production of physically detectable particles by electron microscopy, radioactive labeling, and the assay of virus-specific enzymes. A part of the tissues was homogenized and examined for the ability to transform human embryo cells. Thus far no clear evidence for virus has been found by either physical or biological means.

Significance to Biomedical Research and the Program of the Institute:

Since the viral etiology of cancer has been demonstrated in mice, chickens, hamsters, rats, cats, and some nonhuman primates, it is unlikely that man is an exception. In this project, methods effective in recovering viruses from covert infections in leukemia and sarcoma of animals are systematically being applied to similar tumors of man.

Proposed Course: Research investigations under this contract have been underway for a relatively short time. Effort will continue as described.

Date Contract Initiated: April 27, 1971

Current Annual Level: $159,000

RUSH-PRESBYTERIAN-ST. LUKE'S MEDICAL CENTER (NIH-71-2032)

Title: Studies of Tumor Viruses in Small Primates

Contractor's Project Director: Dr. Friedrich Deinhardt

Project Officers (NCI): Dr. Robert A. Manaker
Dr. Jack Gruber

Objectives: To study selected viruses and virus-induced neoplasia in small laboratory primates, especially marmosets, and to develop the marmoset as a laboratory animal for experimental use in viral oncology.

Major Findings: A simian sarcoma virus (SSV-1) isolated from a woolley monkey was studied. Tumor induction was observed in marmosets inoculated with this virus. The development of focus assay was completed. SSV-1 induced transformation of marmoset fetal lung cells (MFL) grown without agar overlay. Foci of piled up, spindle-shaped cells appeared as early as 4 days after infection, with little or no tendency toward satellite formation; final counts were made 21 days after infection. The titers of SSV-1 in unconcentrated supernatants collected from tumor cell cultures averaged 10^2 focus forming units/ml in untreated indicator cells but pretreatment of the indicator cells with 20 µg/ml of DEAE-dextran for 1 hour before infection increased the titers at least 10-fold. Indicator cells inoculated with dilutions of SSV-1 beyond the endpoint of focus induction also produced C-type virus, and challenge experiments indicated the presence of a 10- to 100-fold excess of an associated, interfering, nontransforming virus. Human embryonic lung cells (WI-38), in parallel assays with MFL, were similarly susceptible to transformation by SSV-1.

Group specific (gs) antigen was prepared in quantity in several batches, and antisera are being prepared in rabbits. Study of relationships of SSV-1 gs antigen to other gs antigens of mammalian and avian origins is near completion. Antigens and antisera were exchanged with Drs. Old and Schaefer for comparative studies.

Investigation of optimal conditions for assay of polymerase of SSV-1 is in progress. Marmoset sera for studies of inhibition of various viral polymerases was sent to Drs. Todaro and Gallo.

The RD-114 cell line from a human rhabdomyesarcoma, received recently from Dr. McAllister, was established in the laboratory. Six marmosets inoculated with viable cells or cell-free supernatants were free of tumor. Studies of the natural distribution of Herpesvirus saimiri (HVS) were continued with the following findings: Virus isolated from 11 of 13 blood samples obtained from healthy squirrel monkeys (SM). These isolates were shown to be antigenically similar if not identical to HVS. By co-cultivation with Vero cells virus was isolated from both whole blood (WB) and lymphocytes separated from WB on Ficoll-Hypaque (FH) gradients (FH-lymphocytes). Attempts failed to isolate virus from FH-cell-pellets (erythrocytes and polymorphonuclear leukocytes) or cell-free extracts of WB, FH-lymphocytes or FH-cell-pellets. The SM isolates were neutralized or reacted positively in immunofluorescence tests with anti-HVS sera but not with antisera to herpes simplex virus or Herpesvirus playrrhinae. Cotton-topped(Saguinus oedipus)and white-lipped (S. fusciollis and S. nigricollis)marmosets, experimentally infected with one isolate, developed a lymphoproliferative disease and survived 18-26 days post-inoculation. Macroscopic and microscopic features of the neoplastic disease were indistinguishable from those produced by the prototype strain of HVS.

The incidence of latent HVS infection in 2 colonies of SM were studied by isolation of HVS from blood obtained from healthy SM and by evaluation of HVS serum antibody levels by indirect fluorescent antibody methods. By co-cultivation of whole blood (WB) or peripheral lymphocytes (PL) with susceptible cells, HVS was isolated from 63 of 139 (45%) SM, but attempts failed to isolate virus from cell-free extracts of WB and PL. HVS was isolated from SM 2 months old but not from 5 younger SM. Only 30% of animals 1 year of age had HVS antibodies, whereas 100% of animals two or more years old had antibodies with peak titers of 1:128. Both of these findings suggest horizontal transmission of HVS early in life. Arrangements have been made to receive young, antibody-negative SM to study the effect of primary HVS infection.

Lymphoid cell lines established from HVS-infected marmosets were further characterized. Six lymphoid cell lines were established and have been serially propagated up to 8 months in cell culture. Four grew in suspension as single cells and clumps of cells, one cell line grew as a monolayer with many free floating cells and one line grew only as a monolayer. Infectious HVS was continually recovered from all the lymphoid cell lines by co-cultivation of the cells with susceptible indicator cells, and several months after the cultures were initiated very small amounts of infectious HVS also was recovered intermittently from supernatants of the cell lines. HVS-associated antigens were demonstrable in 0.1-1% of the cells by indirect fluorescent antibody methods. No specific chromosomal aberrations were observed in the cell lines after 7 months cultivation. Electron microscopic studies and cloning experiments with the lymphoid cell lines are in progress.

Significance to Biomedical Research and the Program of the Institute:

Restrictions in the host range of viruses require that a primate animal be available for study of human virus isolates. The marmoset is an excellent small primate for experimental purposes because twins are born frequently, this species has proven to be responsive to the oncogenic activity of viruses of lower animals and of other primates, it breeds well in captivity. The work accomplished under this contract has provided considerable information, not only on oncogenic RNA viruses, but has been contributing substantially to an understanding of the role of herpesviruses in oncogenic processes.

Proposed Course: The direction of effort described will continue. Studies will concentrate on herpesvirus induced neoplasia and will include RNA viruses isolated from lower primate and human tissues as these become available for this purpose. At present, the leukemia-associated virus of the Gibbon is under investigation preliminary to animal trials.

Date Contract Initiated: March 15, 1962

Current Annual Level: $522,880

RUTGERS UNIVERSITY (NIH-71-2077)

Title: Studies on Genetic Acquistion of Oncogenic Potential by RNA Animal Viruses

Contractor's Project Director: Dr. Robert W. Simpson

Project Officers (NCI): Dr. Michael A. Chirigos
Dr. Robert A. Manaker

Objectives: To determine whether a non-oncogenic RNA animal virus can gain tumor-producing or cell-transforming capability as a consequence of host-induced genetic modification of the viral RNA, by intracellular persistance of incomplete but functionally active viral genetic material, or by induced mutation.

Major Findings: Inactivation of candidate viruses such as vesicular stomatitis virus (VSV) by polyene antibiotics has been exploited as a method for initiating chronic infections at the genetic level. The most active antibiotic found in this system was a water-soluble methyl ester of amphoteracin B which does not degrade virions or produce detectable changes in the viral RNA. Collaborative work done with Dr. D. H. L. Bishop indicates that certain polyenes can replace nonionic detergents in in vitro RNA polymerase reaction mixtures with VSV suggesting that these antibiotics act to alter properties of the viral lipoprotein membrane of intact virions.

Newborn guinea pig heart cells originally infected with VSV inactivated by the compound ethylene iminoquinone (Bayer A 139) have been maintained in vitro for 3 months with no evidence for production of infectious virus.

Persistent infections have been established in HEp-2 carcinoma cells with host-range mutants of VSV that replicate nonpermissively in these cells.

Electron microscopic examination of normal guinea pig cell lines developed in this project has failed to reveal presence of C-type particles or other virus-like particles.

Attempts to induce immunological tolerance to VSV in neonatal guinea pigs were unsuccessful.

Significance to Biomedical Research and the Program of the Institute:

The genetic acquisition of oncogenic potential by RNA viruses is an intriguing possibility and one of great importance, since it is possible that a human cancer virus could be a member of an RNA virus group that normally causes non-oncogenic diseases in man. Although supporting evidence for this phenomenon in RNA viruses is quite scarce, some observations have suggested that ordinarily non-oncogenic viruses may have oncogenic potential. Thus, a thorough investigation of the capacity of non-oncogenic viruses to acquire genetic oncogenic potential is warrented.

Proposed Course: Emphasis will be shifted to attempts to activate latent viral genomes in cells originally exposed to chemically-modified or genetically restricted virus (VSV). Conditional lethal mutants of VSV will be examined for their pathogenesis in guinea pigs and capacity to effect immune paralysis. In vitro studies will be continued to ascertain the spectrum of viral genome expressions in cells harboring non-infectious, virus-specific, informational molecules.

Date Contract Initiated: February 15, 1971

Current Annual Level: $135,758

ST. JUDE CHILDREN'S RESEARCH HOSPITAL (NIH-71-2134)

Title: Studies on the Etiology of Selected Amphibian Tumors

Contractor's Project Director: Dr. Allan Granoff

Project Officers (NCI): Dr. Gary Pearson
Dr. Wilna Woods

Objectives: To determine the role of viruses, particularly herpes-type virus, in the etiology of renal carcinoma of Rana pipiens (Lucke' tumor)

Major Findings: The renal carcinoma of the frog appears free of herpesvirus at ambient summer temperatures. After 2 or more months at hibernating winter temperatures, herpesvirus and intracellular inclusion bodies develop in the renal tumor. The ascites fluid from hibernating tumor-bearing frogs has been found to be rich in Lucke' herpesvirus (LHV). Frog embryos inoculated with herpesvirus-containing ascites fluid develop renal carcinoma. This provides additional evidence for the tumorigenic activity of cell-free herpesvirus and another potential source of virus other than urine and tumor. The contractor's success in carrying out the "in vivo" virus tumor induction assay in his own laboratory now enables him to more easily enlarge the scope of his experiments. A number of cell systems have been tested for susceptibility to LHV using a variety of conditions, but evidence of virus infection has not been detected. Attempts to establish a transplantable Lucke' tumor have been initiated. Preliminary results indicate that more basic information on host vs graft reaction in this system is needed. In preparation for a search for virus-specific products in summer virus-free tumor cells, isotopically labeled RNA from both hibernating and summer tumors and from primary cultures of tumor cells has been prepared. Additionally, isotopically labeled DNA from several tumors has been obtained from frogs active at ambient temperatures.

Significance to Biomedical Research and the Program of the Institute:

Herpesviruses have been associated with the induction of lymphoproliferative diseases in animals and man. In this respect they resemble the RNA leukemia viruses of animals. One of the known RNA viruses, the mouse mammary tumor virus, induces carcinomas of the mammary gland. A parallel activity in regard to epithelial cell transformation is exhibited by the Lucke' virus. It is unknown whether herpesviruses mimic the oncogenic activity of RNA viruses by direct action in converting tissues of both and mesenchymal origin, or whether such activity is indirect, i.e., the herpesvirus infection activates expression of pre-existing RNA tumor virus infection. This project is important in providing for development of an experimental system for more intensive analysis of herpetic infections in neoplastic transformations.

Proposed Course: Effort will be continued to establish a virus-producing system in cell cultures. A major requirement for more intensive study is a reliable source of virus in reasonable quantities. The Lucke' virus will be more completely characterized, and antisera to the virus prepared in homologous and heterologous hosts will be tested for neutralizing capability. Such reagents will permit studies on the "rescue" of the virus from tumor cells, infectivity for different cell systems, and the detection of virus-specific antigens in virus-free cells. Viral DNA and complementary RNA will be prepared for further investigations on the virus-cell relationship. Evidence of C-type RNA viruses in tumor tissue will be sought.

Date Contract Initiated: May 13, 1971

Current Annual Level: $54,485

UNIVERSITY OF TEXAS, M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (PH43-65 604)

Title: Studies on the Relationship of Viruses to Neoplasia

Contractor's Project Director: Dr. Leon Dmochowski

Project Officers (NCI): Dr. Gary Pearson
Dr. Robert A. Manaker

Objectives: To pursue a systematic study of selected human patients with neoplastic disease to establish the possible significance of viruses in the etiology of their tumor.

Major Findings: Indirect immunofluorescence tests were applied to determine the reactivity of sera from cancer patients and their family members with their tumor cell antigens. Antibodies were detected in 50 percent of patients with osteosarcoma and 30 percent of their families which reacted with antigens on their own and other patients' tumor. Sera from normal donors were negative. Some of these sera reacted with antigens on cells of other types of tumor.

Osteosarcoma tissues cultured together with fresh human leukemic bone marrow appeared to undergo a morphological transformation. Supernatent fluids from these cultures induced focal areas of transformation in monolayers of cultured human embryo cells. RNA-dependent DNA polymerase activity was detected in the transformed cells but not in untransformed cells. Similar transformations were observed within co-cultures of other types of sarcomas and leukemic marrow cells. Particulates, detected in these cultures, had a buoyant density of 1.16 and 1.22 gm/cc, contained 50-70 S RNA and had reverse transcriptase activity. Cell-free supernatants from whole human embryo cells transformed in these studies could transmit transforming activity serially to fresh human embryonic cells.

Molecular hybridization studies have shown that the reverse transcriptase of SD-MSV-M (rat bone tumor virus) virions synthesizes DNA species from endogenous viral RNA which represents approximately 85-100% of the viral genome.

The nucleolar antigen in the tumor cells of some patients with melanoma fluctuates with the progression of the disease. Detection of this antigen may indicate changes in prognosis as an aid to early therapy. The antigen can now be localized by ferritin and by peroxidase labeling.

Sera of some patients with breast cancer, of their relatives, and of some normal individuals have antibodies which give cytoplasmic fluorescence with cells of mouse mammary tumors producing type B and type C virus particles. The nature of this reaction is being studied by suitable absorption experiments. About 50 percent of breast cancer patients examined had antibodies which reacted with intracellular antigens in tissues of breast cancers and fibrocystic disease. The presence of particulates in some cultured breast tumor tissue did not correlate with the immunological findings.

A cell line derived from pleural effusion cells from a 5-year-old child with Burkitt's lymphoma (American type) was established and found to produce type C particles. The cell lines, ESP-1, produces sufficient virus for study. The cells react by immunofluorescence with sera from some patients with lymphoma, leukemia, and solid tumors, as well as with sera from a very small number of normal donors. ESP-1 cells also give positive immunofluorescence reaction with sera of the family members of the child from whom the pleural effusion was derived. These cells have HLA patterns of the parents of the child from whom the cells were originally obtained. The type C virus particles from ESP-1 cells contain RNA-dependent DNA polymerase and 60-70 S RNA. Several lines of investigation indicate that the ESP-1 type C virus particles are immunologically distinct from other mammalian type C virus particles, although findings by some investigators suggest immunological relationship between ESP-1 and mouse leukemia virus. Further studies to characterize the ESP-1 virus are in progress.

Significance to Biomedical Research and the Program of the Institute:

Current program requires intensive, systematic investigations on selected human neoplasias to determine possible viral associations with the disease process. The work done on this project complements research in other laboratories and provided a major contribution to overall program.

Proposed Course: Investigations will continue to determine the significance of the transformations induced in human embryo cell cultures by fluids from combined sarcoma-leukemia cell cultures. The ESP-1 virus will be further characterized in collaboration with other laboratories within the Special Virus Cancer Program.

Date Contract Initiated: March 19, 1965

Current Funding Level: $698,700

NCI-NINDS-EMORY UNIVERSITY (NIH-NINDS-72-2301)

Title: Oncogenic Potential of Herpesviruses in Primates

Contractor's Project Director: Dr. John Sever

Project Officer (NCI): Dr. Robert A. Manaker

Objectives: To determine the possible etiological relationship of herpes simplex virus type 2 to cervical carcinoma using the Cebus monkey as an experimental animal system.

Major Findings: This project has recently been initiated. The feasibility of the Cebus monkey for experimental use for studies on cervical carcinoma has been established. Animal holding facilities are being equipped, and the necessary animals are being acquired.

Significance to Biomedical Research and the Program of the Institute:

Seroepidemiological studies suggested that the herpesvirus type 2 strains (HSV-2) infecting human genitalia might be etiologically associated with development of cervical carcinoma. Subsequent studies on UV-irradiated HSV-2 supported this possibility by demonstrating neoplastic transformation of cultured hamster cells exposed to infection. The Cebus monkey has been shown to develop vaginal lesions repeatedly upon exposure to infection by HSV-2. These lesions resemble closely those observed in infected women. This animal provides excellent opportunity to investigate the influence of HSV-2 infection in combination with the sexual activity which appears to predispose to tumor development in humans. As such, this project is most important to overall program.

Proposed Course: The project will be developed as planned. Animals will be observed for 3 years after initial infection.

Date Contract Initiated: March 1, 1972

Current Annual Level: $245,000

Dr. Paul H. Levine, VLLB, Etiology Area, NCI, Chairman
Dr. Ronald B. Herberman, CTIS, LCGBY, GL&C, NCI, Vice-Chairman
Dr. Gary R. Pearson, VBB, NCI, Executive Secretary

THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE (NIH 71-2109)

Title: Anti-tumor Reactivity in Patients with Leukemia and Lymphoma

Contractor's Project Director: Dr. George W. Santos

Project Officer (NCI): Dr. Ronald B. Herberman

Objectives: To perform assays for cellular and humoral immunity against tumor-associated antigens in patients with acute leukemia and lymphoma, with an aim toward using one or more of these assays to monitor immunotherapeutic manipulations. To correlate the results of these assays with each other and with the clinical state of the patients. To search for common antigens in these tumors and to look for immunological reactivity of family members and unrelated individuals to the tumor antigens.

Major Findings: Twenty-one patients with acute leukemia, mainly acute myelogenous leukemia, and three patients with lymphoma have been studied. Only initial testing of the patients against their tumor cells has been performed thus far, because of the prolonged period of chemotherapy. Skin tests with mitomycin-treated cells have given negative results thus far. Mixed lymphocyte cultures with HL-A identical siblings have given positive results in 3 of 4 families. In the cellular cytotoxicity assay, some potitive results against leukemic cells have been obtained with 2 families. The migration inhibition assay has given negative results thus far. A cytotoxic antibody has been found in the serum of a father of a patient with acute myelocytic leukemia and in the sister of two normal adults. This antibody appears to react specifically with leukemic cells.

Significance to Biomedical Research and the Program of the Institute: Clinical, epidemiological and laboratory studies suggest that acute leukemia in man is a virus induced disease. Study of immune reactions to tumor-associated antigens in patients with leukemia and their family members should be useful in evaluating the role of possible oncogenic viruses in human tumors and in providing assays for monitoring attempts at immunotherapy.

Proposed Course: Reactivity to autologous and allogeneic tumor cells will be looked for. Tests will also be performed on family members against remission cells of the patients as well as against leukemic cells. Efforts will be made to further develop the migration inhibition assay, with an emphasis toward performing a direct migration assay. The specificity of the observed antibody reactions and of the cellular reactions will be closely examined.

Date Contract Initiated: May 1, 1971.

Current Funding Level: $110,000

ROSWELL PARK MEMORIAL INSTITUTE (NIH 72-2014)

Title: Stimulation of Immunity Against Tumor by Enzymatically Treated Autologous Cells

Contractor's Project Director: Dr. James F. Holland

Project Officer (NCI): Dr. Paul Levine

Objectives: To develop a vaccine that is effective in the prevention and cure of murine leukemia and to apply these findings to the treatment of human leukemia.

Major Findings: Studies using combination chemotherapy and immunotherapy were developed indicating that neuraminadase-modified leukemia L1210 cells were effective in the treatment of murine leukemia. Groups of mice receiving cyclophosphamide, methotrexate, and BCNU were treated with a single injection of 10^7 vibrio cholera neuraminadase-modified leukemia L1210 cells intraperitoneally. Between 30 and 90 percent of mice were cured by a single dose of neuraminadase treated cells in each of these chemotherapy groups.

The findings obtained with L1210 were applied to spontaneous leukemia occurring in AKR mice. Remission was induced with Vincristine and steroids, and immunotherapy was implemented with neuraminadase treated thymocytes. Approximately 30 percent of the animals were still alive and free of residual disease at 100 days in two experiments, whereas immunization with neuraminadase-treated L1210 leukemic cells was ineffective. In addition, immunotherapy with AKR neuraminadase-treated cells without preceding chemotherapy was ineffective.

Significance to Biomedical Research and the Program of the Institute: In spite of the increasing effectiveness of chemotherapy and the treatment of acute leukemia in humans, relatively few patients have long survivals. Acute myelocytic leukemia, in particular, is a devastating disease with a poor prognosis. The use of immunotherapy to increase the length of survival provides great promise because it enables the individual to use his own host mechanisms to control the disease without the toxic effects from chemotherapy that are required to eliminate the last leukemic cell. The findings from this contract, which uses drug protocols currently in effect in humans, will be directly applicable to the control of human leukemia. The ability of this investigator to achieve better results with spontaneous AKR leukemia, a disease of known viral etiology which is associated with a poor immune response to the disease in the host, indicates that this method for management human leukemia may be successful

Proposed Course: Attention will be given to determining the specificity of the immunotherapy. A correlation between the effectiveness of cell-mediated and humoral factors in control of the disease will be pursued. The treatment protocols necessary to obtain the maximum response from immunotherapy will be evaluated.

Date Contract Initiated: September 15, 1971

Current Funding Level: $135,700

M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (NIH 71-2178)

Title: Immunological Treatment of Human Neoplastic Disease

Contractor's Project Director: Dr. Joseph G. Sinkovics

Project Officer (NCI): Dr. Paul H. Levine

Objectives: To develop an in vitro test system for cell-mediated immunity using a standard cell line as antigen; to use this test system in a longitudinal follow-up of cancer patients and relate the in vitro findings to the clinical course; to apply the principles obtained in the in vitro studies to the immunological manipulation of the patient with the goal of improving the prognosis in these diseases.

Major Findings: In the first year of contract performance, the contractor has established a number of cell lines from tumors which have morphological and growth characteristics of tumor cells. He has improved the testing procedure and is now in a position to carefully monitor cancer patients over a period of time. Initial studies suggest that the test is able to detect tumor-associated antigens and that the evaluation of a variety of immunological factors, including cytotoxic and blocking factors in the patient's sera, can be correlated with the clinical course of the disease in most patients.

Significance to Biomedical Research in the Program of the Institute: The definition of tumor-specific antigens in human tumors provides a basis upon which to identify a human tumor virus. Studies characterizing tumor-specific antigens are important to the program of the Institute not only as an initial phase in the identification of possible human tumor viruses, but in the establishment of test systems for monitoring the patient's immunological response to tumor-associated and viral antigens. This contract is developing an in vitro test system to identify tumor-specific antigens in tissue culture lines. The cell lines will be prime candidates to investigate the possible presence of a human tumor virus and will also provide a standard reagent to use in immunotherapy of human cancer.

Proposed Course: Concentrate on the specificity of the newly developed in vitro immunity test with particular attention to human sarcomas. Continue to apply the test system in longitudinal studies of human cancer patients with a clearer definition of the operation of lymphocytes, blocking factors and unblocking factors in the patient's blood.

Date Contract Initiated: June 29, 1971.

Current Funding Level: $111,520

THE RESEARCH FOUNDATION STATE UNIVERSITY OF NEW YORK (NIH 71-2137)

Title: Application of Immunotherapy Proven Curative for Epidermal Tumors to other Types of Malignant Disease

Contractor's Project Director: Dr. Edmund Klein

Project Officer (NCI): Dr. Charles Boone

Objectives: To extend the application of skin sensitizer therapy proven curative for skin tumors to other types of cutaneous neoplasms as well as to deep-seated tumors of other organs.

Major Findings: The following patients demonstrated at least partial and frequently complete regression of their tumors following therapy with skin sensitizers: 8 of 14 patients with nodular basal cell carcinoma, 4 of 5 patients with mycosis fungoides, 3 of 4 patients with cutaneous reticulum cell sarcoma, 1 of 2 patients with multiple cutaneous and subcutaneous lesions of malignant melanoma, 1 of 2 patients with cutaneous metastases of mammary carcinoma, 1 of 2 patients with advanced carcinoma of the vulva. Collaborative studies were initiated with Dr. Robert Huebner to determine if evidence of immunity to any virus-associated antigens could be detected in patients whose tumors appear to be determined at least in part by genetic factors. In addition, collaborative studies with other SVCP investigators have been developed to compare immunity with specific tumor-associated antigens with immunity to the skin sensitizers used in therapy.

Significance to Biomedical Research and the Program of the Institute: The Principal Investigator is one of the few persons obtaining clinical cures of cancer with a type of therapy which appears to have an immunological basis. Using a technique that has proven to be successful in the field, it is possible to evaluate the mechanisms involved and apply them to other human tumors. In addition to providing data useful for the early diagnosis and treatment of cancer, this contract also provides materials from patients that are well characterized so that the unusual genetic and immunological findings can be applied to studies being carried on by other areas in the Special Virus Cancer Program.

Proposed Course: In the coming year, emphasis will be placed on the immunological mechanisms responsible for the successful therapy of cutaneous neoplasms with sensitizers. In addition, the role of genetics will be pursued in collaboration with other SVCP investigators. A correlation of the non-specific immune response with immunity to specific tumor-associated antigens will be expanded applying *in vitro* tests of cellular and humoral immunity as well as skin testing.

Date Contract Initiated: May 25, 1971.

Current Funding Level: $86,995

TRW SYSTEMS GROUP (NIH 70-2200)

Title: Viral Antigens and Anti-Viral Antibodies

Contractor's Project Director: Dr. Norman Weliky

Project Officer: Dr. Vincent W. Hollis

Objectives: To purify, separate, and characterize the antigens in known and candiate oncogenic viruses and the corresponding anti-viral antibodies.

Major Findings: A method using immunoadsorbents was developed for absorbing all detectable antibodies to mouse serum components from rabbit antiserum prepared by injecting mouse serum. The conditions for removing antilipoprotein antibodies were also determined. A study of the optimum conditions for absorbent preparation and for batch absorption indicated three things: (1) Sephadex G-200 worked better than G-50, G-25, or agarose; (2) for activation, 500 mg of cyanogen bromides per 100 mg G-200 worked better than reduced quantities of cyanogen bromide; (3) preparations made by coupling activated G-200 to mouse serum at pH11 were better than those coupled at pH10. Additional experiments established that goat and rabbit antihuman serum could be cleared of antihuman serum antibodies by an analogous procedure, using immunoadsorbents prepared with human serum and with human serum lipoprotein. The monkey anti-Rauscher plasma virus antiserum provided by the Project Officer was absorbed with immunoadsorbents and Bionetics Research Laboratories confirmed the absence of antimouse serum antibodies in the absorbed serum. HA titer to RLV was retained.

Significance to Biomedical Research and the Program of the Institute: Antigens cross-reacting with murine and feline leukemia viruses have been reported in human leukemic cells. This contract, by purifying and separating all the antigens associated with a known animal leukemia virus (Rauscher Leukemia Virus), will provide direct evidence to confirm or refute the existence of the cross-reacting viral antigens in animal and human tumors.

Proposed Course: Isolation and purification of murine viral antigens will be continued. Experiments will also be undertaken to isolate and purify EBV-associated antigens.

Date Contract Initiated: June 15, 1970

Current Funding Level: $121,464

ROBERT B. BRIGHAM HOSPITAL (NIH 71-2172)

Title: Tumor-specific Transplantation Antigens in Solid Tumors

Contractor's Project Director: Dr. John David and Dr. W.H. Churchill

Project Officer (NCI): Dr. Gary Pearson

Objectives: To search for and characterize tumor-specific antigens in human tumors using the macrophage migration inhibition assay.

Major Findings: Experiments designed to search for evidence of production of MIF by human lymphocytes stimulated by autologous tumor have been pursued. Modifications of the human MIF assay have been made to reduce the number of false negative assays. Assays have been attempted on materials from 21 patients with a variety of tumors (colon, breast, lung, lymphosarcoma, bladder) and completed in 14 instances. No evidence of MIF release in response to human tumor antigen has been obtained. The line 1 guinea pig hepatoma system has been used to develop a target cell cytotoxicity system. These cells were labelled with 3H-thymidine for 24 hours and then exposed to immune cells. The percent kill was determined by measuring the residual radioactivity after 48 hours. Specific cell kill in excess of 50 percent was measured by this method. Blocking antibody activity could not be demonstrated using serum from animals nearly moribund from line 1 tumor. Selective accumulation of basophils has been demonstrated at sites of reaction of either line 1 or line 10 tumors by specifically immunized animals. Accumulation of basophils has also been found at sites of reaction to line 1 extract in the skin of line 1 immune animals but not at sites of reaction to line 10 extract. Hypertonic KCI extracts of line 1 tumor inhibited the migration of specifically sensitized cells.

Significance to Biomedical Research and the Program of the Institute: In vitro assays for the degree of cellular immunity in human cancer patients are urgently needed so that (1) anti-tumor immune responses may be followed during the course of the disease and (2) large numbers of human tumors can be screened for tumor distinctive antigens. Successful application of the MIF, blocking antibody, and cell cytotoxicity tests in this laboratory has shown that in vitro assays of the guinea pig system can be useful in correlating cytotoxic and morphologic parameters in human studies. Extracts made by methods that were useful in human skin testing also work in KCI-macrophage migration test in guinea pigs. It is expected that improvements of the MIF assay and other tests with the same extracts will improve comparative studies in human and animal tumors. Better defined cross-reacting tumor antigens will provide leads for the morphological type of tumor that is most likely to have a virus causation.

Proposed Course: (1) To continue to use the MIF assay to look for evidence of cellular immunity in man to tumor antigens. KCI extracts of human tumors will be evaluated as one possible source of tumor antigen; (2) the mechanism of tumor cell kill in vitro will be studied in the newly developed target cell cytotoxicity system.

Date Contract Initiated: March 12, 1969.

Current Funding Level: $74,000

UNIVERSITY OF CALIFORNIA, LOS ANGELES (NIH 72-2008)

Title: Cellular and Humoral Immunity to Tumor Antigens

Contractor's Project Director: Dr. Paul Terasaki

Project Officer (NCI): Dr. Ernest J. Plata

Objectives: To establish the number of distinct tumor-specific antigens which exist in human tumors utilizing cellular and humoral immunity tests. To study the epidemiology of cellular immunity to tumor antigens. To test for humoral antibodies on cancer patients by blocking cellular immunity and direct reaction with tumor cells. To monitor the degree of cellular immunity during chemotherapy. To study the linkage of HL-A antigens to susceptibility to tumors.

Major Findings: In the first year of operation, the Takasugi-Klein technique was automated and an objective machine reading was developed. Collaborative studies with other SVCP contracts were developed which have already resulted in two manuscripts which were submitted for publication. Particular areas of importance were: (1) HL-A antigens and cancer patients: The degree of correspondence in the frequency of 25 separate HL-A antigens was remarkably similar for 1100 cancer patients, with tumors of the bladder, breast, prostate, lung, colon, and cervix, in comparison to the frequencies in normal control populations. Some differences which were significant, however, were the excess of HL-A1 and W18 (Te58) in Hodgkin's disease and of HL-A1 in cervix cancer. A higher incidence of Te66 in lung cancer was also of interest. More effort is now being made to subdivide the patients with tumors and increase the numbers of patients with rarer tumors. (2) Serum antibody studies: Sera from 288 cancer patients were first screened for HL-A antibodies by testing with lymphocytes from 80 random donors. They were then tested against a panel of tissue cultured tumor cells by a new plating inhibition test. Using this microtest, 2,459 combinations of serum and tumor cells were examined. Some 6% of the combinations tested yielded positive reactions. The specificity of the antibodies are being investigated. (3) Cellular immunity: A total of 63 cultured lines have been reacted with lymphocytes from over 1000 cancer patients in the micro cell-mediated test. Conditions of the test, particularly the amount of media was thoroughly studied. From the results obtained, lymphocyte mediated immunity appears to be only partially specific.

Significance to Biomedical Research and the Program of the Institute: This contract allows the application of findings in tumor immunology and genetics to large scale population studies. As evidence for tumor-specific antigens develops, population studies allow the identification of diseases which give an epidemiological pattern suggestive of an environmental agent. By determining the immune status of unaffected individuals and family members of cancer patients to tumor-specific antigens, it is possible to utilize epidemiology to select tumors which have the greatest (and poorest) chances of being caused by a virus. The studies on HL-A antigens are particularly significant because of the ability to separate diseases which

have a genetic predisposition from those which do not. The identification of people who are at high risk of developing cancer will allow specific actions to be taken in regard to diagnosis, prevention and treatment on a more manageable level.

Proposed Course: During the next fiscal year, the major effort will be focused on demonstrating the specificity of the immunological reactions that are occurring in the cancer patients. Specificity of the serological reactivity will also be analyzed using cytotoxicity and blocking studies. The role of genetics in human tumors will be pursued in conjunction with the immunological and epidemiological studies.

Date Contract Initiated: July 12, 1971.

Current Funding Level: $273,600

CHILDREN'S HOSPITAL OF PHILADELPHIA (PH 43-66-477)

Title: Interference and Immunoflurescence Studies with Cell Lines Derived from Leukemias and Lymphomas

Contractor's Project Director: Dr. Gertrude Henle

Project Officer (NCI): Dr. Paul Levine

Objectives: (1) To evaluate antibody patterns to EBV related antigens, i.e., the viral capsid antigens (VCA), early antigens (EA), and the D and R components of the EA complex in various EBV-associated diseases and controls. (2) To devise additional test procedures for humoral and cell mediated immune responses in EBV-associated diseases. (3) To investigate methods for improved propagation of the EB virus.

Major Findings: EBV-induced early antigens (EA) were found to be more disease-associated than the viral capsid antigen (VCA), in that patients with infectious mononucleosis (IM), Hodgkin's disease (HD), chronic lymphocytic leukemia (CLL), and Burkitt's lymphoma (BL), were more likely to have antibodies to EA even with low VCA titers. Normals generally did not have antibodies to EA, even with high VCA titers.

Exposure of Raji or RPMI-64-10 cells (free of detectable EBV-related antigens) to EBV derived from the HR1-K subline of P3J cells, led essentially only to abortive infections in invaded cells; that is, early antigens (EA) were synthesized but not viral capsid antigens (VCA). This permitted seperate determination of antibodies to EA and VCA in indirect immunofluorescence tests, using acetone-fixed cell smears of abortively infective Raji cells for anti-EA, and of EB3 (or HR1-K) cells for anti-VCA. Anti-VCA titers usually exceed anti-EA titers by several dilution steps.

In the course of examination of hundreds of sera for antibodies to the EA complex, two different patterns of immunofluorescence were observed: (1) Diffuse staining (D) of the nucleus and cytoplasm of infected cells; and (2) Restricted staining (R) of masses in the cytoplasm. The D component resisted methanol or ethanol fixation but was rapidly and completely destroyed by proteolytic enzyme treatment of acetone-fixed cell smears. The R component was denatured by methanol fixation, but was more resistant to proteolytic enzymes than D. Abortively infected Raji cells with R antigen always contained D. However, the D containing cells did not always contain R. The D antigen was found early after infection in the nucleus only. In time it was found in the cytoplasm and thus filled the whole cell. The R antigen developed entirely in the cytoplasm.

Data confirmed that there were three diseases which were regularly associated with high antibody levels to EBV-related antigens: IM, BL, and NPC. In these cases, anti-VCA titers usually persisted at nearly constant levels, whereas considerable changes in anti-R, anti-D, or both were observed frequently in the course of the disease. Approximately 80% IM and NPC patients have high anti-D titers which usually decline with cessation of the disease or when patients go into prolonged remissions. Anti-EA, particularly anti-R, has some prognostic significance in BL patients who have been successfully brought into remission by Chemotherapy. Those who maintain or develop high levels of anti-EA are more prone to have relapses than patients with no or declining titers of anti-EA. The reason why the anti-EA response is dominantly or solely of the anti-D type in some disease and of the anti-R type in other diseases is presently unexplained.

Several thousand sera are being titrated for antibodies to various EBV-related antigens from patients with Burkitt's lymphoma (BL), nasopharyngeal carcinoma (NPC), Hodgkin's disease (HD), lymphosarcoma (LSA), and several other malignant and non-malignant diseases. Results of these studies are being tabulated and complete analysis is being postponed until sufficiently large numbers of patients in each category have been examined.

Significance to Biomedical Research and the Program of the Institute:
A major effort of the SVCP has been to study EBV involvement in the etiology of human malignancies as BL and NPC. This contract characterized several EBV-related antigens and investigated their relationships with many malignant and non-malignant diseases. The identification of two EA antigens has indicated the posibility that different strains of EBV may be present or that if one strain is involved, the immune response may be of diagnostic or prognostic importance.

Proposed Course: Continuation and completion of the above studies and particular attention to development of an assay for cell-mediated immunity to EBV.

Date Contract Initiated: January 1, 1966.

Current Funding Level: $115,150

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NIH 70-2076)

Title: Seroepidemiology Studies of Nasopharyngeal Carcinoma and Burkitt's Lymphoma

Contractor's Project Director: Dr. G. Blaudin de The

Project Officer(NCI): Dr. Robert Depue

Objectives: To determine whether there is an etiological relationship between a herpes-type virus, the Epstein-Barr Virus (EBV), and Burkitt's lymphoma; to study groups with high and low risk of nasopharyngeal carcinoma (NPC) with the aim of evaluating the possible interrelationship of genetics, environment, and viruses (especially EBV) in the etiology of NPC.

Major Findings: (1) Uganda: A five-year prospective study was initiated to investigate the status of African children relevant to EBV antibody levels prior to the development of Burkitt's lymphoma. (2) Hong Kong - Singapore: More than 7,000 blood samples have been collected from normal individuals to evaluate the distribution of EBV antibodies and HL-A patterns in groups at high and low risk for nasopharyngeal carcinoma. Analysis of a cross-sectional collection of sera from 2,096 individuals in the Hong Kong area indicate that EBV titers remain at a constant level from ages 2-30, but that after the age of 30, the proportion with titers greater than 1:160 increases in both males and females. Higher titers were found in the young Singapore population where approximately 20 percent of the pupulation between ages 19-29 had EBV titers greater than 1:160. (3) In Lyon, an attempt was made to standardize the EBV testing between Hong Kong, Singapore and Lyon. Studies performed at Lyon on a French population indicated a bio-modality of EBV titers with a gap from ages 10-40. A significant difference in antibody pattern was noticed from village to village in the French survey. Some villages had a substantial proportion of titers of 1:10 or below, whereas other villages had virtually no individuals with low titers. A soluble antigen from Pope's QIMR-WIL line derived from a leukemic patient reacted in high titer with NPC sera but not with BL sera.

Significance to Biomedical Research and the Program of the Institute: High EBV-antibody titers have been associated with nasopharyngeal carcinoma and Burkitt's lymphoma, but an etiological relationship has not been established. The prospective study initiated in Uganda should be able to

detect whether Burkitt's lymphoma is the result of a new infection with EB virus in an older child who has somehow managed to escape infection with EBV at an early age, and it should also be able to test an alternative hypothesis that Burkitt's lymphoma requires persistant reinfection with EBV in order to develop. The Far Eastern studies will be able to concentrate on a multi-factorial analysis of possible etiological co-factors in the etiology of a tumor with clear population clustering. The correlation of data resulting from the HL-A typing, EBV studies, and case control studies could identify factors involved in the etiology of this disease. The serological findings of higher titers in the older Hong Kong population indicated that active reinfection with EBV occurs in an area where there is a high incidence of NPC, and is therefore in support of the reinfection theory of EBV oncogenicity. The findings of EBV antibody differences by city in France are important because of the time-space clustering and drifting of Burkitt's lymphoma. Geographical variation in EBV status in consistant with the hypothesis that EBV causes Burkitt's lymphoma. The findings of a soluble antigen that differentiates between sera with Burkitt's lymphoma and nasopharyngeal is one of two important tests that are able to distinguish between high EBV-associated antibody levels, the other being the finding of the diffuse type of early antigen in NPC by the Henles while the restricted is more often found in Burkitt's lymphoma.

Proposed Course: Continued collection of data and materials in the Hong Kong, Singapore and Uganda areas will be pursued as above. Standardization of the EBV test will be assisted by cooperation between standardization laboratories in Lyon and the United States.

Date Contract Initiated: January 1, 1971.

Current Funding Level: $523,615

CENTER FOR DISEASE CONTROL (VCL-42)

Title: Etiologic Studies of Leukemia and Related Diseases Occurring in Unusual Epidemiologic or Genetic Situations.

Contractor's Project Director: Dr. Clark W. Heath, Jr.

Project Officer (NCI): Dr. Adi F. Gazdar

Objectives: (1) To perform epidemiologic and virologic studies of leukemia and related illnesses (cancer, congenital defects) occurring in unusual epidemiologic or genetic circumstances. (2) To maintain leukemia case surveillance programs in selected population areas in the United States.

Major Findings: Out of approximately 100 cases of leukemia and lymphoma brought to the attention of the CDC, 41 cases associated with unusual circumstances were selected for epidemiologic and laboratory study. The studied cases consisted of 24 community clusters, 10 multiple case families, 5 human-animal case associations, and 2 instances of marital leukemia/lymphoma.

A total of 35 cell lines from leukemia/lymphoma cases have been permanently established, screened by electron microscopy, tested for the presence of oncogenic and non-oncogenic viruses, immunoglobulin production and mycoplasma contamination by various techniques and preserved in liquid nitrogen. In the past year, 60 specimens have been collected for cell culture work, and about 150 serum specimens have been obtained, aliquoted and stored.

Thirteen cell lines derived from patients have been cross-tested for FA reactivity against the donor's serum and 2 cell lines were also tested against sera collected from relatives and contacts, with negative results.

Cell cultures, mostly from multiple case family situations have been supplied to several investigators in the SVCP. Eighteen cell cultures have been tested at the NCI for reverse transcriptase activity, with negative results.

Significance to Biomedical Research and the Program of the Institute: This contract deals with epidemiologic and genetic studies on cases of human leukemia and attempts to ascertain and clarify the etiology of human luekemia through intensive investigations of selected cases. Since clustering and genetic defects are associated with the easier detection of virus in animal systems, this contract provides specimens of particular importance to SVCP investigators because of the greater chance of identifying a human tumor virus.

Proposed Course: The contractor will continue to identify and study cases of particular interest to SVCP. In addition, a program for a systematic evaluation of the relationship between infectious mononucleosis and lymphoma will be developed.

Date Contract Initiated: July 1, 1967.

Current Funding Level: $150,000

THE UNIVERSITY OF MINNESOTA (NIH 69-2061)

Title: Tumor specific Transplantation Antigens in Solid Tumors: Evaluation of the Immune Response

Contractor's Project Director: Dr. Charles R. McKhann

Project Officer: Dr. Ronald B. Herberman

Objectives: To study events in the immune response to tumor antigens by assays for lymphocyte stimulation, cytotoxicity by immune cells, blocking antibody, and cytotoxic antibody. To study the effect of clinical state on the immune response. To perform studies on immunological manipulation of experimental tumors.

Major Findings: A heterologous antiserum to mouse plasma cells has been developed. This serum suppressed formation of antibody to sheep erythrocytes. It was found to have some inhibitory effects upon the growth of transplantable mouse tumors.

Lymph nodes of tumor bearing mice were found to produce larger amounts of immunoglobulin than those of control mice.

A serial study of cellular immune reactivity of patients to tumor-associated antigens is now in progress.

Significance to Biomedical Research and the Program of the Institute: Study of immune reactions to tumor-associated antigens on human tumors is an important part of the AVCP. Studies of immunological manipulation in animal model systems may provide information leading to successful immunotherapy in man.

Proposed Course: (1) Continue studies on inhibition of antibodies to tumor antigens by the anti-plasma cell serum. (2) Continue serial study of immune reactivity in patients.

Date Contract Initiated: April 14, 1969.

Current Funding Level: $130,011

CHILDREN'S HOSPITAL OF THE DISTRICT OF COLUMBIA (NIH 72-2071)

Title: Immunologic Reactivity of Pediatric Cancer Patients

Contractor's Project Director: Dr. Sanford Leiken

Project Officer (NCI): Dr. Edward Leonard

Objectives: To determine if children with cancer react immunologically to autologous neoplastic cells and to serve as a resource of tumor tissue from children with neuroblastoma, rhadomyosarcoma, Wilms tumor and hepatoma.

Major Findings: This is a newly funded contract and there are no findings to be reported.

Significance to Biomedical Research and the Program of the Institute: The Special Virus Cancer Program has been concerned with the problem of whether or not cancer patients possess specific tumor immunity. Evidence has been obtained by others using in vitro tests that neuroblastomas from different patients share tumor antigens. Clinical immunological and epidemiological studies indicate that neuroblastoma may be a tumor of viral origin. This contract represents the first attempts to verify in vivo antigenic relationships among neuroblastomas from different patients. Tests for cellular immunity using skin testing and in vitro techniques, when combined with virological and biochemical tests, will be of great importance to studies on the viral etiology of neuroblastoma. In addition, if reactivity is detected it may be possible in the near future to increase the immune response by the patients to the point where an adverse effect on the tumor and accompanying beneficial effect for the patient would be obtained.

Proposed Course: The contractor will concentrate on preparation of tumor cell antigens for studies which will include: (1) measurement of in vivo delayed cutaneous hypersensitivity reactions to antigen preparations; and (2) in vitro lymphocyte transformation and colony inhibition tests.

Date Contract Initiated: February 1, 1972.

Current Funding Level: $70,000

GEORGE WASHINGTON UNIVERSITY (NIH 72-3251)

Title: Clinical Tests for Immune Responses to Separate Soluble Membrane Antigens Which Appear to be Tumor-Specific

Contractor's Project Director: Dr. T. Crandall Alford

Project Officer (NCI): Dr. Ronald Herberman

Objectives: To study the immunological reactivity of tumor patients to antigens on their neoplastic cells by skin testing and other in vitro methods and to correlate these results with the clinical course of the disease.

Major Findings: This is a newly funded contract and there are no findings to be reported.

Significance to Biomedical Research and the Program of the Institute: The potential findings of common antigenicity of tumors would provide strong indirect evidence for viral etiology. This contract should: (1) allow the study of several immunological parameters in patients with neoplastic diseases; (2) provide information on the immunogenicity of soluble antigens and their potential usefulness in immunotherapy; (3) contribute information regarding common antigens in tumors of different individuals with the same disease; (4) provide materials for a correlation of tumor-associated antigens with biochemical and virological searches for a human tumor virus.

Proposed Course: The contractor will test soluble extracts from tumors and selected patients will be inoculated with these extracts to determine if the tumor specific antigens are immunogenic. Materials from these patients will be studied in collaboration with biochemists and virologists within SVCP.

Date Contract Initiated: April 1972

Current Annual Level: $84,955

PROGRAM MANAGEMENT SEGMENT
Dr. J. B. Moloney, ASDVO, ET, NCI, Chairman
Dr. Louis R. Sibal, OASDVO, ET, NCI, Executive Secretary

UNIVERSITY OF NORTH DAKOTA (PH43-66-8) GRAND FORKS, NORTH DAKOTA

Title: Quantitative Studies on the Transmission of Feline Oncogenic RNA Viruses and Selected Herpesviruses by Certain Bloodsucking Arthropods

Contractor's Project Director: Dr. Robert G. Fischer

Project Officer (NCI): Dr. George J. Burton

Objectives: To determine whether certain oncogenic viruses can be transmitted from infected animals to healthy susceptible animals by bloodsucking arthropods. Specific objectives are: (1) To determine virus levels in arthropods which have fed on experimentally infected donor animals of known titer. (2) To determine whether the disease caused by each oncogenic virus can be transmitted biologically, i.e. by transfer of the virus via the salivary glands of the infected arthropod. (3) To determine the quantitative distribution of the virus among various organs and tissues in those arthropods demonstrating suitably high virus levels. (4) To determine whether the disease caused by each virus can be transmitted mechanically through interrupted feeding, or by transfer of the virus on the legs and body of the arthropod.

Major Findings: (a) Friend leukemia virus (FLV).

In collaboration with Drs. W. Turner and G. Burton, N.I.H., the X-C test was used to determine whether the Friend leukemia virus (FLV) complex ingested by the stable fly is altered in such a way that subsequent introduction of this insect-virus mixture into an animal system results in splenomegaly without splenic foci. The findings are consistent with the hypothesis that a "helper" activity (LLV) may be degraded more rapidly than SFFV. A low percentage of flies are capable of supporting SFFV plus helper activity for at least 14 days. An investigation to determine possible SFFV and/or helper virus (LLV?) activity in the stable fly following a single FLV meal was conducted. A study with Dr. R. Steeves, Roswell Park Memorial Institute, indicated a gradual loss of both SFFV and helper viruses. FLV infection rate studies have been conducted in other fly and mosquito species. It was found that 90-95% of the mosquitoes tested had each ingested enough FLV to infect one weanling BALB/c mouse.

(b) Feline leukemia virus (FeLV) and Feline Sarcoma virus (FSV): No virus particles have been observed in various organs of cat fleas or mosquitoes which fed on the FeLV- or FSV-infected donor cats. The fate of FSV in the cat flea, fed successive infective meals, is currently being evaluated.

(c) Herpes saimiri virus (HVS): Arthropod transmission of Herpesvirus saimiri (HVS) which induces reticulum cell sarcoma in marmosets has been investigated with the collaboration of Dr. F. Deinhardt, Presbyterian-St.

Luke's Medical Center, Chicago, and Dr. G. Burton, N.I.H. They included initial extrinsic incubation studies of HVS in relation to the yellow fever mosquito, Aedes aegypti; the malaria mosquito, Anopheles quadrimaculatus; the cat flea, Ctenocephalides felis; and the stable fly, Stomoxys calcitrans. HVS was only isolated from the midgut of Aedes and Anopheles mosquitoes, cat fleas, and stable flies up to 6 hrs. In an investigation of the possibility of transmission from an HVS-infected marmoset to a susceptible recipient during interrupted feeding activity of the stable fly, four of the six marmosets showed no apparent signs of disease after one year.

(d) Marek's disease virus (MDV): Marek's disease transmission studies are being conducted with the collaboration of Drs. J. Frankel of Life Sciences Inc., and Dr. G. Burton, N.I.H. During this first triannual period, an interrupted feeding-natural association experiment was conducted. Stable flies, Aedes mosquitoes, and triatomid bugs were allowed to partially feed on two MDV infected chickens. One hour later, the insects were allowed to complete feeding on varying numbers of both one-day-old L.S.I. and conventional Wallace chicks. No positive transmissions have occurred to date.

(e) Murine cytomegalo virus (CMV) (Henson strain). This cytomegalovirus has been supplied by Dr. D. Henson of N.I.H. Results of three experiments with stable flies indicate that CMV is viable in this insect for at least 3-4 days.

Significance to Biomedical Research and the Program of the Institute:

Viruses, which are obligate parasites, may be transferred biologically or mechanically by arthropod vectors. Mosquitoes, biting flies, fleas, ticks, and mites may suck blood from both man and domestic animals (including birds) closely associated with man. It is, therefore, important to investigate whether oncogenic viruses present in infected animals can be transferred to man. If such a vector is found, measures can be taken to control or prevent contact between the vector and human or animal hosts.

Proposed Course:

(1) Since the Herpesvirus saimiri appears to have no viability in the yellow fever mosquito, malaria mosquito, cat flea, and stable fly beyond 6 1/2 hours, only interrupted feeding transmission experiments will be pursued next year. (2) Insect transmission experiments will be continued using Marek's disease virus, murine cytomegalovirus (Henson strain), and feline oncogenic viruses, with mosquitoes, flies, fleas, and other arthropods. (3) Additional species of mosquitoes, flies and fleas will be used as test insects in these transmission experiments.

Date Contract Initiated: October 27, 1965

Current Contract Level: $25,000 (It is planned to terminate this contract after Dec. 31, 1972, since all objectives will probably have been met by then.)

UNIVERSITY OF MIAMI SCHOOL OF MEDICINE (PH43-67-1187) CORAL GABLES, FLORIDA

Title: Immunization Studies on Avian Leukosis and Related Problems

Contractor's Project Director: Dr. M. Michael Sigel

Project Officer (NCI): Dr. Gary Pearson

Objectives: (1) Measurement of the duration of immunity induced by live virus; (2) Further assessment of the specificity of protection induced by immunization with live virus; (3) Search for humoral factors other than neutralizing antibodies; and (4) Investigations of the several parameters of cell-mediated immunity.

Major Findings: (1) Their initial observations on protection against Rous tumor induction were extended to include various combinations of immunizing and challenge viruses of subgroups A, B, and C of the avian leukosis-sarcoma complex. It was demonstrated that protection against tumor induction by viruses of all 3 subgroups (A, B, C) could be achieved by immunization with a single line leukosis virus of any of the 3 subgroups. Furthermore, this protection was independent of humoral antiviral neutralizing antibody. Taken as a whole, their work seems to provide an indirect demonstration of a TSTA or VISA on the natural host system for avian leukosis and sarcoma viruses. (2) The duration of protection elicited by RAV-1 immunization was at least 9 months. (3) Immunization of chickens 2 weeks of age afforded better protection against a challenge dose RSV(RAV-1) than immunization of 14 week old chickens. (4) Inactivated virus (B-propriolactance and formalin) failed to elicit protection against RSV(RAV-1). Utilizing the in vitro blastogenic reaction, it was found that leucocytes sensitized to virus of one subgroup were also responsive to antigen(s) associated with another subgroup. The development of the blastogenic response began slowly and reached its peak 4-6 weeks after immunization which coincided with the period necessary for immunity to tumor induction to develop in immunized birds. (5) Soluble virus-induced surface antigen (VISA) was prepared from SR-RSV transformed hamster cells (MSR-5) by the method of Mann et al. The soluble extract was capable of blocking the serum mediated cytotoxic reaction against both allogeneic (clone-2) and syngeneic (MSR-5)SR-RSV transformed hamster cells. Preliminary attempts to prepare soluble RSV induced surface antigen using a KCL solubilization procedure have been started in order to determine the most suitable and efficient method for isolation of VISA.

Significance to Biomedical Research and the Program of the Institute: The overall goal of the SVCP is the control of cancer. From the immunological viewpoint, it would be extremely important to clarify the issue as to the possible existence of a transplantation antigen and its role in the disease process. Basic studies are also needed on immunity in chickens in order to understand fully the immunological implications in avian tumors, and possible application to mammals.

Proposed Course: (1) Experiments on immunization of Japanese quail, which

are free of endogenous gs antigen and helper factor, are now underway. (2) Attempts to achieve protection against sarcoma viruses in chickens and quail through immunization with a subgroup E virus are planned. (3) A further effort to immunize with a killed virus preparation, using hydroxylamine-inactivated virus is also planned. (4) Major emphasis will be placed on further work with the putative VISA isolated from RSV transformed tumor cells and on the relative blastogenic potential of chicken lymphocytes from immunized birds as correlated with tumor immunity.

Date Contract Initiated: June 23, 1967

Current Funding Level: $121,000

UNIVERSITY OF WASHINGTON (NIH-NCI-72-2037) SEATTLE, WASHINGTON

Title: Immunological and Transplantation Studies on Dogs with Cancer for Detection of an Oncogenic Virus-Carrier State

Contractor's Project Director: Dr. Rainer Storb

Project Officer: Dr. Gary Pearson

Objectives: (1) To set up procedures for studies of immune competence in dogs with lymphosarcoma and other tumors before, during, and after treatment; (2) To monitor closely those dogs undergoing relapse for biochemical or other expression of an oncogenic virus; (3) To set up immunological assays for investigation of tumor-specific antigens of canine tumors; (4) To carry out allogeneic marrow grafts in dogs with tumors as a means of preventing recurrence of leukemia.

Major Findings: This contract was awarded November 1, 1971. Because of delays in the delivery of some equipment that was vital for the initiation of the work, progress has been hampered. However, they have succeeded in starting some experiments with (a) 5 dogs with lymphomas, (b) 1 with Waldenstrom's disease, and (c) 4 dogs with other malignant tumors. Three types of studies have been carried out in dogs with lymphoma or Waldenström's disease: (1) One of two normal dogs was given lymphosarcoma cells immediately after irradiation. Both dogs were then infused with previously stored autologous bone marrow followed by methotrexate therapy. A dramatic increase in the concentration of DNA polymerase containing particulates in the plasma of the recipient dog that was given lymphosarcoma cells before irradiation was noted confirming previous findings. (2) One tumor dog was given 1200R of total body irradiation followed by infusion of phenotypically histocompatible marrow from a normal donor dog and post-grafting treatment with methotrexate. A dramatic regression of all tumor-bearing lymph nodes occurred within a few days following irradiation. This dog is now in apparent complete remission with a successful hemapoietic graft 55 days after the transplant. (3) One dog has been subjected to total body irradiation followed by infusion of previously stored autologous bone marrow in order to evaluate the effects of total body irradiation alone on the treatment of lymphoma. Two dogs with

other malignant tumors were given total body irradiation followed by infusion with either phenotypically histocompatible marrow from a normal dog or autologous marrow. The hemapoietic grafts were successful in both dogs and some tumor regression was noted.

Significance to Biomedical Research and the Program of the Institute: The major goal of the SVCP is the prevention or control of human neoplasia. The projected immunotherapy is aimed at providing the clinician with an additional modality of therapy which might eradicate those tumor cells which for one reason or another survive chemotherapy. Another goal of this project is to attempt to determine whether tumor-specific antigens exist in canine neoplasms. If specific transplantation antigens are detected and prove to be cross-reactive, indirect evidence will then be available for the viral etiology of canine lymphosarcoma.

Proposed Course: Until the arrival of the dog cages necessary for the *in vivo* investigations, efforts will be directed to establishing the necessary *in vitro* laboratory tests for searching for tumor-specific antigens on canine tumor cells. When the cages become available, the biochemical studies on the significance and nature of the DNA polymerase containing particulates found in the plasma of irradiated dogs will be continued. Investigations on the immunotherapeutic effects of bone marrow grafts will also be continued.

Date Contract Initiated: November 1, 1971

Current Funding Level: $160,000

UNIVERSITY OF MIAMI SCHOOL OF MEDICINE (NIH-70-2211) MIAMI, FLORIDA

Title: Studies of the Rat Mammary Tumor Derived Virus (RMTDV or BV)

Contractor's Project Director: Dr. Victor V. Bergs

Project Officer (NCI): Dr. Michael A. Chirigos

Objectives: (1) To determine whether purified C-type particle preparations of RMTDV will induce leukemia or other malignancies when administered to conventional and germ-free rats under natural and immunologically altered conditions of the host; and (2) comparative studies of the biochemical and biological properties of rat C-type viruses (BV-1, R-35, and WF-1) of different origin.

Major Findings: Studies in collaboration with NCI staff have thus far revealed that the C-type virus, which emerged spontaneously in a 2-year old line of "normal" W/FU rat embryo cells (WF-1 virus), is antigenically related to the rat C-type viruses (BV-1 & R-35) isolated from rat mammary tumors. The WF-1 virus produces cell alterations in REL cultures. It does not exhibit characteristics common to murine C-type viruses (gs-1 & gs-3 antigens, and the XC reaction).

From purification studies of the BV-1 and WF-1, diaflo ultrafiltration,

centrifugation in discontinuous and continuous sucrose gradient, it was found that the virus was distributed in 2 peaks with average densities of 1.16 and 1.20 gm/cm^3, respectively. Infectivity studies showed that the greatest occurred in the 1.20 gm/cm^3 peak. Tests were performed to substantiate this observation through the use of ^{3}H-uridine-labeling, possible contamination with cell debris or mycoplasm. Results from these studies rule out these possibilities. The procedures employed were examined by the use of ^{3}H-uridine labeled RSV(RAV-1), which demonstrated a radioactive peak at a density range of 1.15 to 1.16 gm/cm^3 in sucrose.

The BV-1 virus produces cytopathic effects in BHK-21 cells when infected cells are incubated with medium containing 10% newborn calf serum. BV-1 virus, (harvested from the chemically infected REL cell line, BV-40), infects REL and BHK-21 cells resulting in the formation of plaques. The plaque assay will allow for cloning and further purification of BV-1 virus.

Studies were completed on viral RNA of the BV-1 and WF-1 viruses. In both viruses, using established extraction procedures, the sedimentation velocity of the RNA was estimated to be 25 to 30S, with approximately 10% of the radioactivity in a region corresponding to 4-10S RNA. RSV(RAV-1) was used as a control in these studies and the expected profiles of 65-70S and 4-10S RNA were obtained. The 25-30S RNA component is suggestive of (a) degradation of the RNA, which is not expected since nuclease inhibitors are used; however, RSV(RAV-1) RNA is not degraded under the same conditions; (b) a subunit structure in the viral genome which is unlike the subunit structure of other C-type viruses; (c) ribosomal RNA of either procaryotic or eucaryotic origin. Experiments are underway to clarify these points.

Significance to Biomedical Research and the Program of the Institute:

Although rats are highly susceptible to spontaneous cancers as well as to the induction of cancers by chemicals and irradiation, and C-type viruses similar to those which cause cancers in other species have been found in rats by electron microscopic studies, no such virus of rat origin has yet been proven to cause a cancer in this species. In addition to the important possibility of having a new laboratory model in another species for guiding approaches to the human virus-cancer problem, the proposed studies would also provide an important test of the Huebner hypothesis that C-type viruses are the determinants of most cancer in all species, including rats and humans for which oncogenic viruses have not yet been demonstrated.

Proposed Course: (1) Further purification studies on BV-1 and WF-1 viruses. (2) Studies on viral RNA. (3) Comparative studies of the biochemical and biological properties of rat C-type viruses (BV-1, R-35, and WF-1) of different origin. (4) Attempts to potentiate the WF cell agent by serial *in vivo* passage of the cells, accompanied by inoculation of rats with cell-free extracts of the tumors.

Date Contract Initiated: June 23, 1967

Current Funding Level: $51,167 (This contract terminates June 30, 1972).

UNIVERSITY OF INDIANA, INDIANAPOLIS, INDIANA (NIH-69-2048)

Title: Characterization of the Twiehaus Agent of Avian Reticuloendotheliosis

Contractor's Project Director: Dr. Alvin S. Levine

Project Officer (NCI): Dr. Michael A. Chirigos

Objectives: (1) To test transmissibility of Twiehaus virus. (2) To determine whether this agent acts as an immunogen. (3) To determine what mitochondrial alterations occur with Twiehaus agent pathogenicity. (4) To determine the presence of reverse transcriptase in this agent.

Major Findings: In day-old cockerels all routes of inoculation (intracranial, intramuscular, intracardiac, intranasal, intraperitoneal, subcutaneous, and via the alimentary canal) were capable of inducing deaths from reticuloendotheliosis virus (REV). From 15 moribund cockerels sampled individually, infectious REV was obtained from oral-nasal washings, gut washings, feather-shaft follicle tips, and skin containing feather follicles, but not from feather dander. Antibody to REV was detected in the serum of contact cagemates. None of the normal control birds, housed separately in the same room, were serologically positive. In spite of the presence of infectious virus in moribund birds and the serologic conversion of the contact mates, horizontal transmission of the disease symptoms was not demonstrated. The results suggest that close contact is required for horizontal transmission of the virus, and that contact with REV in this manner either fails to produce disease or produces a subclinical form.

White Leghorn birds were hyperimmunized with purified, sucrose-banded reticuloendotheliosis virus (REV) of three types: (1) REV from tissue culture (REV-T), (2) sodium dodecylsulfate (SDSO-treated REV-T, and (3) REV purified from liver-spleen homogenates (REV-L). These groups of birds, when challenged with a 10% gravity-settled homogenate of RE-specific liver-spleen homogenate, demonstrated protection against REV at levels of 97%, 77% and 100%, respectively, in the order of hyperimmunizing material listed above. When these same birds were further challenged with cloned strains of Rous sarcoma virus, SR-RSV-B and RSV(RAV-1), REV-T-hyperimmunized birds were protected against these agents at levels of 100% and 93%, respectively. Birds hyperimmunized with SDS-treated REV-T were protected against SR-RSV-B and RSV (RAV-1) at levels of 100% and 29%, respectively. REV-L-hyperimmunized birds were protected against RSV(RAV-1) at a level of 62%. RSV(RAV-1)-immunized birds were not protected against challenge with REV.

In preliminary studies of liver mitochondria from reticuloendotheliosis virus (REV-T) infected chickens, gross alterations were observed in the oxidative and phosphorylative behavior of these organelles. Respiratory activity (Q_{O2}) was greatly diminished and phosphorylative activity, as estimated by state 4-state 3 transitions, in a polarographic apparatus was absent. Succinoxidase activity was enhanced after incubation in an isotonic medium, suggesting large amplitude swelling. Mitochondrial preparations were marked by the appearance of a yellow fluorescent pigment which has not been characterized to date.

It has been postulated that the pathogenicity of REV-T is due to induced emboli. The underlying molecular mechanism may be at the level of the mitochondria, as suggested by these data.

RNA-dependent DNA polymerase activity was originally demonstrated in the virions of avian and murine leukosarcoma virus complexes and has been extended to 27 isolates of oncogenic RNA viruses. Employing published procedure, the Tweihaus agent was studied for the presence of the transcriptase. The in vitro DNA polymerase assay used followed the method reported by S. Spiegelman. The results obtained show that the RNA-dependent DNA polymerase is associated with the Tweihaus agent and is similar to that present in the avian leukosarcoma viruses and in other oncogenic RNA viruses.

Significance to Biomedical Research and the Program of the Institute:

The avian reticuloendotheliosis virus is a leukemogenic virus prevalent in many flocks of chickens, and is said to be transmitted both horizontally and vertically. The biologic characteristics and the understanding of the chemical, physical and immunological nature of the agent may be applicable to the possible role of viruses as etiologic agents in human neoplasia. Of equal importance is the determination of whether oncogenic viruses of animals have the capabilities to replicate and induce neoplastic diseases in man. The avian viruses must be evaluated for this potential.

Proposed Course: (1) Attenuation of REV(T) for vaccine strain(s). (2) Immunological comparison of REV(T) with other avian tumor viruses. Immunological study of antigen preparations from purified preparations of REV. (3) REV(T) with Marek's disease herpesvirus in vitro and in vivo to determine if interference or potentiation results. These studies are being carried out in collaboration with Life Sciences, Inc. (4) Characterization of REV polymerase(s) and the effect of specific REV(T) antibodies on polymerase activity.

Date Contract Initiated: May 8, 1969

Current Contract Level: $77,485

ATOMIC ENERGY COMMISSION, OAK RIDGE NATIONAL LABORATORY (FS-7)

Title: Biophysical Instrumentation Development and Cancer Anatomy Program

Contractor's Project Director: Dr. Norman G. Anderson

Project Officer (NCI): Dr. Charles W. Boone

Assistant Project Officers (NCI): Dr. Robert A. Manaker
Dr. Timothy O'Connor

Objectives:

1. To investigate the immune reaction to cancer and to develop rapid monitoring assays for this purpose.

2. To determine the re-expression of fetal, embryonic, or placental antigen or isozymes in tumors, and to develop reagents and immunological tools for early cancer detection and therapy based on these antigens.

Major Findings:

1. Demonstration of circulating antibodies cytotoxic to SV40 hamster tumor cells after immunization with human embryonic cells.

2. Development of methods for isolating tumor antigens from tumor cells in tissue culture.

3. Establishment of a screening laboratory for screening human sera for fetal isozymes, antigens and low molecular weight indicator substances.

4. Modification of a cytofluorograph for direct computer data processing.

5. Development of interim fluorescence standards for quantitation of fluorescent binding to cells.

6. Discovery that a large fraction of the circulating anti-Burkitt cell antibodies in convalescent Burkitt patients and in infectious mononucleosis are absorbed out by fetal cells, and that they will also bind to SV40 transformed hamster cells, providing strong support for the concept that many of the antigens appearing in tumors, including human ones, are re-expressed embryonic components.

7. A symposium and workshop on Fetal and Embryonic Antigens in Cancer was held in Oak Ridge in May 1971 and will appear in December of this year. A second one was held in February 1972. Arrangements for rapid publication have been completed.

8. Heavy emphasis is being placed on the characterization of so-called "blocking" components in the serum of cancer patients and tumor-bearing animals.

Significance to Biomedical Research and the Program of the Institute: The development of better methods for characterizing immunological response to cancer and for detecting tumor antigens are closely associated with the elucidation of the viral causation of cancer in man. In the event a human cancer virus is authenticated, immunological methods and concepts now being used to detect cancer viruses will be used diagnostically and to monitor therapy.

Proposed Course: This contract will continue to operate in two research areas: 1) the development of better instrumental methods for detecting cancer antigens and 2) the elucidation of fetal antigens to cancer antigens.

Date Contract Initiated: July 1, 1962.

Current Contract Level: $650,000.

MELOY LABORATORIES (NIH-72-2020)

Title: Cell Biology Facility: Mechanisms of the Immune Response to Squamous Cell Carcinoma, Adenocarcinoma and Fibrosarcoma in the Mouse; Experimental Immunotherapy.

Contractor's Project Director: Dr. Kenneth Blackman

Project Officer (NCI): Dr. Charles W. Boone

Objectives: To elucidate the mechanisms of the humoral and cellular immune response to different kinds of cancer in the mouse. To develop improved *in vitro* and *in vivo* assays for detecting tumor specific antigens and antibodies. To develop systems of immunotherapy.

Major Findings: Cell-free homogenates of fibrosarcoma induced either SV40 virus or methylcholanthrene will immunize animals against tumor transplant challenge if the cells are first infected with influenza virus. The immunogenic principle in the homogenate is membrane-associated and is still active when formalin-fixed. This influenza virus enhancement of immunogenicity does not occur if egg-grown influenza virus is simply mixed with the tumor homogenate.

A precise radioactive footpad assay for delayed hypersensitivity to tumor cells in the mouse was developed with the use of tumor-immune animals and cell lines produced by the contract. The anti-tumor cell reactivity can be adoptively transferred to normal mice by inoculating them intraperitoneally with spleen cells from tumor-immune mice. Tumor-bearing animals do not show delayed hypersensitivity to tumor after the tumor reaches a certain size.

Twenty-three new transplantable tumor lines of methylcholanthrene-induced fibrosarcoma, twenty of mammary adenocarcinoma, and fifteen of methylcholanthrene-induced squamous cell carcinoma of the skin were developed. All of the tumor lines have been carried through three or more transplant generations. All of the fibrosarcomas and some of the adenocarcinomas possess tumor specific transplantation antigens as determined by tumor-graft rejection assay.

The growth of SV40 induced tumor cells is completely inhibited by admixing spleen cells from tumor-immune animals with the challenge inoculum of tumor cells. Titration experiments show that as few as ten million spleen cells are effective.

Splenectomy has been found to augment resistance to tumor transplant challenge.

Significance to Biomedical Research and the Program of the Institute: The ability to augment the immunogenicity of cell-free tumor homogenates by prior infection of the tumor cells with influenza virus provides a way to isolate immunologically effective tumor transplantation antigens from cell-free homogenates of human tumors so that the patient may be immunized against his own tumor without the drawbacks and risk associated with the inoculation of x-irradiated live cells. Research on the mechanisms of the immunological response to tumor will provide ways of manipulating the immune system to the advantage of the cancer patient both in diagnosis and in therapy.

Proposed Course: The relationship of cell surface structure to tumor-host interaction will be included in future studies.

Date Contract Initiated: August 20, 1971

Current Contract Level: $335,000

ATOMIC ENERGY COMMISSION (NIH-NCI-E-[FS-13])

Title: Co-carcinogenesis Program

Contractor's Project Directors: Dr. F. T. Kenney
Dr. G. D. Novelli
Dr. M. G. Hanna
Dr. R. L. Tyndall
Dr. R. W. Tennant

Project Officers (NCI): Dr. James T. Duff
Dr. Timothy O'Connor

Objectives: In January 1963 an interagency agreement was established between the National Cancer Institute and the Oak Ridge National Laboratory for carcinogenesis studies. During the past 3 years the agreement has been funded by both the Carcinogenesis and Viral Oncology (SVCP) Program Areas of NCI. Only the SVCP funded portion is reported here. The broad objectives are (1) to study the interaction of RNA tumor viruses with the host immune mechanism (Hanna); (2) to conduct investigations on the biochemical mechanisms involved in the initiation of cancer (Novelli); (3) to provide fundamental understanding of the mechanisms by which gene expression is regulated in mammalian cells (Kenney); (4) to investigate the commonality between embryogenesis and oncogenesis and its possible significance in the control of cancer (Tyndall); and (5) to study the functional and antigenic properties of cells infected or transformed by murine RNA tumor viruses, and the nature of the interactions between cells, viruses, and carcinogens (Tennant).

Major Findings: An improved method of isolating reverse transcriptase from spleens infected with Rauscher leukemia virus has been developed. The quantity of reverse transcriptase isolated from the microsomal fraction of a gram of infected spleen is equivalent to that obtained from 0.4-0.8 ml of a 10X virus concentrate from mouse plasma, indicating the superiority of

infected spleen as a source of the enzyme.

The optimal reaction conditions for reverse transcriptase from Rauscher leukemia virus (RLV) apparently differ from those for the same enzyme isolated from avian myeloblastosis virus (AMV), regardless of the template used. Other significant differences are: the AMV transcriptase is larger than the RLV enzyme, and is more resistant to inhibition by poly(U) or Poly(G); the purified RLV enzyme is quite stable, whereas purified AMV form is very labile. For the RLV enzyme, optimal conditions differ markedly depending on the template used in the reaction mixture. For example, Mg^{++} is the preferred ion in the DNA-dependent system, while Mn^{++} is much less active for DNA-dependent activity but is absolutely required for RNA-dependent activity. The polymerase has a tenfold higher affinity for ribopolymers than for deoxyribopolymers.

Recent studies with 90% pure, Type A particles from mouse plasma-cell tumors support the idea that these particles do not contain reverse transcriptase.

Electron microscopic examination of myeloma tumor cells from BALB/c mice previously infected with RLV and bearing transplanted myelomas revealed budding Type C particles, suggesting that myeloma blast cells, like immunoblasts in the spleen, are very susceptible to RLV infection.

Polyadenylic acid [poly(A)] reproducibly inhibits Moloney virus replication in cultured mouse cells, but only when added before or during the first 4 hours of infection. Poly(U) and poly(C) were inactive while poly(G) was cytotoxic. The inhibition by poly(A) is not due to any observable effect on growth and division of the host cells. This finding supports the interpretation that the effect of poly(A) on leukemia virus replication is due to the specific inhibition of viral DNA synthesis.

The restrictiveness of human cells for mouse leukemia virus replication was investigated by cell fusion techniques. Virus synthesis could not be detected in human cells or in human-mouse heterokaryons, but did occur in mouse cell synkaryons. These results suggest that the nonpermissiveness of human cells for Moloney leukemia virus (MoLV) is dominant. Five cloned cell lines of human-mouse hybrids, containing less than the full complement of human chromosomes, were also tested for permissiveness to MoLV, and were found to be receptive to virus synthesis, suggesting that some human gene(s) act to inhibit replication of the virus. Attempts to correlate this nonpermissiveness of human cells with a specific chromosome are underway.

In a previous study, immunization with mouse fetal cells suppressed the growth of both plasma cell tumors and spleen cells infected with Rauscher leukemia virus, suggesting a cross-reactivity between fetal cells and the two types of tumor. To establish true cross-reactivity, it was necessary to demonstrate the reciprocal of the above (that is, suppression of fetal cells after immunization against the tumors). An *in vivo* assay for quantitating immunization against injected cells was developed, based on the colony-forming units (CFU) test in the spleen. With this assay, it was shown, for the first time in an *in vivo* system, that fetal cells are indeed suppressed in mice immunized with plasma cell tumors. This

demonstration, coupled with the reciprocal finding is compelling evidence that fetal cells and plasma cell tumors have common cell-surface antigens.

Significance to Biomedical Research and the Program of the Institute: This effort is focused on the phenomenon of carcinogenesis, considering this as a fundamental biological problem with common features whether the carcinogenic stimulus be a chemical, or radiation, or a virus. Special attention, however, is given to particular areas such as carcinogenesis and cocarcinogenesis in the respiratory tract and the role of both Type C viruses and cellular control mechanisms in murine leukemias.

Proposed Course: Continue to develop a concerted, interdisciplinary research program in the central aspects of both chemical and viral carcinogenesis.

Date Contract Initiated: July 1, 1963 (Viral Oncology Funding: July 1, 1970)

Current Contract Level: $1,000,000

UNIVERSITY OF WISCONSIN (NCI-E-72-2022)

Title: Role of RNA tumor viruses and related genetic information in induction of tumors by chemicals.

Contractor's Project Directors: Dr. Charles Heidelberger
Dr. James Miller

Project Officers (NCI): Dr. Roy Kinard
Dr. George Todaro

Objectives: To induce neoplasia in mice and rats in vivo and transformation of mouse and rat cells in vitro and determine the degree of viral activity in this process by immunological techniques, DNA-RNA hybridization and tests for polymerases.

Major Findings: Breeding colonies of 13 inbred strains of mice and two inbred strains of rats have been established to serve as a source of all the test materials to be used under this contract. The following mouse strains are now in production: C57Bl/6, Balb/c, 1, 129, AKR, C58, NZB/Bl, C3Hf/Bl, DBA/2, C3H/An, A, R111, and GR. The inbred rat strains are W/Fu and BN. Mice of these strains are regularly being provided for the chemical induction of tumors. Groups of each of these strains have been injected subcutaneously with either 250 µg of 3-methylcholanthrene (MCA) in 0.1 ml of trioctanoin or with the solvent alone.

The mouse breeding colony has also been utilized as a source of cells for in vitro carcinogenesis studies. Six lines of cells have been obtained from the prostates of individual C3H mice; 3 from anterior prostates, and 3 from ventral prostates. Selected clones derived from these lines are now being tested for malignant transformation using MCA, MCA-epoxide, and N-methyl-N'-nitro-nitrosoguanidine (MNNG).

Lines of mouse embryo cells similar to the original 3T3 Swiss mouse embryo cell line have been established from C3H and AKR mice. These lines have characteristics similar to the original 3T3 line -- in particular, a low saturation density. Clones derived from these lines are now being tested for chemical transformation. A 3T3 line will also be initiated from C57Bl mice for comparative transformation experiments.

Significance to Biomedical Research and the Program of the Institute: The exhaustive yet inconclusive search for oncogenic viruses in human tumors, and in many kinds of tumors in other animals, has turned attention toward the detection of viral genetic information in the form of RNA or DNA or both, which codes for oncogenic RNA viruses or portions thereof. This project is designed to assess the types of tumors in laboratory animals in which viral information is important to the induction of malignancy by chemicals. The objective is to provide a guide to the solution of etiology of human tumors, which is one of the prime targets of the SVCP.

Proposed Course: Continuation as described.

Date Contract Initiated: September 1, 1971

Current Contract Level: $249,500

PUBLIC HEALTH RESEARCH INSTITUTE OF THE CITY OF NEW YORK, INC. (NCI-E-72-2028)

Title: Study of Cell Surface Alterations Induced by RNA and DNA Viruses.

Contractor's Project Director: Dr. Thomas Benjamin

Project Officers (NCI): Dr. George Todaro
Dr. Roy Kinard

Objectives: To investigate the relationship between cell surface alterations induced by RNA viruses and those induced by DNA viruses using several oncorna viruses and non-transforming mutants of polyoma virus.

Major Findings: Eight cell lines including 3T3, 3T12 and sarcoma virus transformed lines, have been transferred from Dr. Todaro's laboratory successfully and are being checked for possible selection of variants. This contract is new and no other results have been obtained.

Significance to Biomedical Research and the Program of the Institute: The long range goal of the research program of this laboratory is an understanding of how oncogenic viruses overcome cellular growth controls. Ultimately, an understanding of the mechanism of neoplastic transformation by viruses will depend on progress in two areas: (1) the identification and characterization of those viral gene functions which are essential to the transformation process, and (2) the determination of how and where these essential viral genes interact with the cell and with factors which normally operate in regulating cell growth. The proposed contract will allow Dr. Benjamin to continue and extend his impressive work on this problem and will foster collaboration with other NCI and SVCP workers.

Proposed Course: Continuation to achieve the objectives described.

Date Contract Initiated: December 3, 1971

Current Contract Level: $36,800

BAYLOR COLLEGE OF MEDICINE (NCI-E-72-2058)

Title: Development of Suppressor Mutants of 3T3 Mouse Cells.

Contractor's Project Director: Dr. Thomas Caskey

Project Officer (NCI): Dr. George Todaro

Objectives: To develop suppressor mutants of 3T3 mouse cell lines

and use these to investigate genes involved in maintaining transformed state in cell cultures.

Major Findings: None reported. The contract has been in effect too short a time to expect any results.

Significance to Biomedical Research and the Program of the Institute: Understanding of the mechanism of action of tumor viruses will require an understanding of the specific genes of the viruses that are responsible for transforming a normal cell into a tumor cell. For these studies, viral and cellular mutants will be essential. The systems of suppressor mutation that Dr. Caskey proposes to develop have unique advantages. They are simpler, less expensive, and produce "absolute" mutants. The project under this contract will provide extremely valuable reagents for the whole SVCP.

Proposed Course: Continuation to achieve objectives described above.

Date Contract Initiated: January 15, 1972

Current Contract Level: $60,500

UNIVERSITY OF ILLINOIS AT THE MEDICAL CENTER (NCI-72-2031)

Title: Studies on the Molecular Mechanism of Carcinogenesis by Oncogenic Viruses.

Contractor's Project Director: Dr. Giampiero di Mayorca

Project Officer (NCI): Dr. George Todaro

Objectives: Development of a system of mutants of MSV by isolation and characterization of temperature sensitive mutants and of lethal mutants for transformation of mouse, hamster, rat sarcoma viruses; identification of the viral proteins responsible for transformation by polyoma and mouse sarcoma viruses; and studies of the mechanisms and kinetics of transformation in cell cultures.

Major Findings: None reported. This contract has been in effect only a few months; the laboratories are only now becoming really operational.

Significance to Biomedical Research and the Program of the Institute: This project will make available important information regarding the molecular mechanism by which viruses cause tumors. In addition, it will develop the technology necessary for the development of vaccine from human tumor virus, if and when such viruses will be discovered.

Proposed Course: Continuation to achieve objectives described.

Date Contract Initiated: December 9, 1971

Current Contract Level: $220,420

BIONETICS RESEARCH LABORATORIES (NIH-69-2160)

Title: Support Services for the Special Virus Cancer Program.

Contractor's Project Director: Dr. Robert C. Y. Ting

Project Officer (NCI): Dr. George Todaro

Objectives: To provide a laboratory that will collect, process and test specimens from human and animal sources suspected of containing virus associated antigens or antibodies, and to provide other virology, immunology or cell culture services as required.

Major Findings: Services and resources provided in close collaboration with NCI investigators during the past year include: (1) biochemical studies of cell growth regulation with Dr. Todaro; (2) attempts to isolate a human cancer virus with Dr. Bassin; (3) tests for EBV antigens for Dr. Levine; (4) immunological tests of leukemia patients, including studies of twins, for Dr. Levine; (5) CF tests for gs antigens for Dr. Hellman; (6) membrane antigen preparation from human tissue for Dr. Herberman; (7) collection of familial cancer sera and histories for Dr. Fraumeni; (8) tissue and serum bank for Dr. Levine et al; (9) American Burkitt registry and follow-up; and (10) data processing with Dr. Waggoner.

When abortively transformed cells containing SV40 genome were re-infected with SV40, they had a lower rate of transformation than cells without the genome; thus, the presence of SV40 did not confer immunity.

Fetal thymus cells of dogs were cocultivated with irradiated human sarcoma cells. The dog cells showed degeneration and transformation (chromosome analysis now being done).

Rhesus cell cultures infected with Mason-Pfizer virus showed evidence of transformation and caused regressing tumors when subsequently inoculated into newborn rhesus monkeys.

Cellular immunity studies of leukemia patients, using lymphocyte cytotoxicity and cytotoxicity inhibition tests, suggest that cells of such patients possess leukemia-associated antigens and that a widespread antigen system may be operative in human and animal tumors.

Significance to Biomedical Research and the Program of the Institute: This contract laboratory provides an opportunity for a systematic,

large-scale effort to detect viruses and/or viral antigens in human tumor materials (particularly leukemias and sarcomas), using tissue culture, immunological, biochemical and EM techniques. This is a major objective of the SVCP.

Proposed Course: It is proposed that this contract will continue to supply the necessary supportive services required to meet the needs of the SVCP.

Date Contract Initiated: June 27, 1969

Current Contract Level: $800,000

MELOY LABORATORIES (NCI-E-72-2006)

Title: Spontaneous and Virus-induced Neoplastic Transformation.

Contractor's Project Director: Dr. John E. Verna

Project Officers (NCI): Dr. George Todaro
Dr. Roy Kinard

Objectives: To study spontaneous and virus-induced neoplastic transformation by the following methods or projects: (1) development and characterization of 3T3 and 3T12 lines from inbred mouse strains; (2) radiation-virus co-carcinogenesis; (3) elucidation of the differences between the normal and transformation prone human cells; (4) characterization of MSV transformed Balb/c 3T3 cells and nonproducer cells; (5) development of assay systems for use in tumor virology; (6) biochemistry of enzymes in tumor viruses; and (7) to search for C-type and B-type RNA viruses in human cells.

Major Findings: The Balb/c and NIH/Swiss embryo cell lines have provided excellent model systems for the study of the effects of tumorigenic viruses both in vitro and in vivo. These cell lines continue to be supplied to numerous investigators throughout the world, and have become the standard cell lines for biochemical and biological investigations of cellular growth control mechanisms.

Human fibroblast cells from patients with a variety of diseases which have been shown to be associated with a high incidence of tumor formation are tested for their susceptibility to transformation by SV40 virus. More than 1200 individual specimens have been cultured for testing. The mechanism which results in the increased susceptibility of certain cells to SV40 transformation appears to be related to a step in SV40 infection which occurs after virus adsorption but prior to the complete uncoating of the virus.

The isolation and characterization of temperature sensitive mutants of viruses associated with the murine leukemia-sarcoma complex is

being done in an effort to characterize the gene(s) responsible for transformation and tumorigenicity. Virus assays performed by focus formation of the murine sarcoma virus and by the SC plaque formation test of the murine leukemia virus have been adapted to microtiter procedures. This modification allows the screening of large numbers of potential mutants in a more efficient manner.

Infection and transformation of human cells with SV40 DNA has been established. Different forms of DNA are being tested to define the smallest piece of viral information required for biologic activity.

Cell lines known to release MSV and MuLV continue to be employed as model systems for the study of virus-induced transformation and tumorigenesis. Methods developed in this laboratory led to the successful isolation and identification of a rat-tropic sarcoma virus, M-MSV-O, which possessed a different host range and serologic characteristic than the Moloney sarcoma virus genome.

Human cells derived from normal tissue and malignant tissue of a wide variety of pathological types are being examined by a variety of biochemical techniques. The primary biochemical technique employed is the RNA-dependent DNA polymerase or reverse transcriptase assay. Tests have been developed to distinguish viral enzyme from normal cellular enzymes. Reverse transcriptase assays provide an extremely sensitive measurement of viral expression in normal cells and in cells transformed by murine oncogenic viruses.

It has been possible to detect enzyme activity in normal as well as viral transformed cells. Normal human fibroblasts have been shown to contain polymerase activity. Since normal cells, as well as tumor cells, contain enzymatic activity, further purification was necessary to make the distinction possible. Antibody to known murine viral polymerase has been prepared and shown to be able to inhibit one enzyme from animal tumor cells. Such antibodies do not affect normal cellular enzymes. Thus, it is possible to isolate and identify a viral specific enzyme in virus-producing cells.

Antisera to purified enzymes have also allowed preparation of antisera to a specific viral antigenic component such as the group specific antigens of C-type virus. Such antisera have been prepared and employed for the detection of viral group specific antigens in the ESP-1 human cell line. Expanded research efforts have resulted in the development of methods which greatly increase the sensitivity of detecting viral RNA-dependent DNA polymerase using synthetic templates. The tumor viruses are partially solubilized and polymerase activity which is associated with the internal core of the virus is quantitatively assayed.

Sera from animals possessing tumor induced by murine-virus were found to contain antibody to the polymerase of the murine virus. Antibody against this purified viral enzyme has now been made in rabbits. These

antibodies cross-react and inhibit the viral polymerases of several mammalian C-type RNA viruses. The antisera does not cross-react with the enzymes of the avian tumor viruses nor of the mouse mammary tumor virus. It is expected that similar techniques will be useful in identifying a possible human viral enzyme. Extensive screening of viruses for polymerase activity has been performed to determine whether the polymerase is specific for tumor viruses. This has led to the discovery that visna virus and foamy viruses also contain the polymerase activity. Syncytium-forming viruses of several species including the primate, bovine, hamster, and feline have been found to contain an RNA-dependent DNA polymerase.

Significance to Biomedical Research and the Program of the Institute: These studies offer the possibility of determining the sequence of events that occur when a normal cell is converted into a neoplastic cell, suggest a possible simple test for "cancer proneness" in man which would permit recognition of a "high risk" group for cancer in the human population and provide further information at the cellular and molecular level on the "oncogene theory" of cancer.

Proposed Course: Effort on this contract will remain essentially the same.

Date Contract Initiated: May 25, 1965

Current Contract Level: $1,757,000

UNIVERSITY OF MINNESOTA (NCI-E-71-2261)

Title: Tumor Viruses in Immunological Deficiency Diseases of Man.

Contractor's Project Director: Dr. Robert A. Good

Project Officers (NCI): Dr. George Todaro
Dr. Roy Kinard

Objectives: To clarify the role of immunologic surveillance, cellular and humoral factors and genetic factors in protection against neoplasia; to search for viruses in patients with premalignancy and on immunosuppressive therapy.

Major Findings: New methods for evaluation of the clinical status of patients with immunodeficiency syndromes and malignancy have been developed. These laboratories can carry out efficiently all of the immunological testing recommended for immunodeficiency patients by the World Health Organization Expert Committee.

Successful explantation of a very high percentage of human tumors has provided material for other SVCP contractors for immunologic and virologic analysis. In nine months of the first contract year they

have successfully explanted 132 tumors, and of these, 54 have been sent to Meloy Laboratories and are available to other contractors on request. Of special interest is a cell strain derived from a patient with an embryonal rhabdomyosarcoma which is currently in its 15th passage.

In the past nine months, skin biopsies have been obtained from additional high cancer risk immunodeficient patients bringing the total number to 33. To date 15 of these have been tested. Cell strains from 14 to 15 patients were found to have low or normal susceptibility to SV40.

Successful explantation of a high percentage of tumors has provided material for evaluation of cell-mediated and humoral immune response of patients to their own as well as histologically identical tumors. In addition to confirming the presense of cell-mediated immunity directed against tumor cells in patients with neuroblastoma and rhabdomyosarcoma, they have demonstrated cell-mediated immunity and serum mediated "blocking" of the cellular response in one patient with lymphosarcoma and another with malignant teratoma.

A new direct culture method for analyzing phytohemagglutination responses of circulating leukocytes which promises to permit quantitation of the number of responding T-cells has been developed and is being tested.

The facilities for evaluating the immunologic status of patients are functioning and operative. The number of patients with unusual immunologic diseases who are available to Dr. Good and on whom he already has extensive immunological data is unequaled.

Significance to Biomedical Research and the Program of the Institute: Knowledge of the immunological capability of humans and their response to viral oncogenes and to viral induced tumor antigens is of great importance to the development of effective means of human cancer control. The project under this contract will provide information and materials from carefully selected patients suffering from immunodeficiency diseases, plus research collaboration between the project officer and his staff and one of the nation's outstanding immunological research teams.

Proposed Course: Continuation with expansion to include study of viral transformation of bone marrow cells and study of effects of nuclear transfer in the Lucké renal tumor of frogs.

Date Contract Initiated: May 13, 1971

Current Contract Level: $275,880

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NCI-E-71-2149)

Title: Studies of Leukemia Virus DNA Polymerase.

Contractor's Project Director: Dr. David Baltimore

Project Officers (NCI): Dr. George Todaro
Dr. Roy Kinard

Objectives: To characterize the enzyme, its product, its mechanism of reaction, and formation of viral RNA during infection.

Major Findings: Using polyribionucleotides as templates, complementary primer was necessary to initiate DNA synthesis. Using poly(A) as a template for the DNA polymerase, the amount of poly(dT) synthesis was proportional to the amount of added template. The best primers were oligodeoxyribonucleotides such as oligo(dT) as a primer for poly(A). Polyribonucleotides were in general much better templates than polyribodeoxynucleotides.

The endogenous reaction involves the copying of the 60S-70S RNA found in the virion. The initial reaction product formed when the virion DNA polymerase copies the endogenous viral RNA consists of small pieces of DNA attached to the 60S-70S RNA. The DNA can be released from the bulk RNA by procedures which disrupt hydrogen bonds. The density of the product is not that of a free DNA but that of a covalently-bonded DNA-RNA hybrid. This finding, which was made both with mouse leukemia virus and avian myeloblastosis virus, indicates that the primer for the endogenous reaction is an RNA molecule.

The globin messenger RNA or, more strictly, the 10S RNA from rabbit reticulocytes polyribosomes, was the best template for the DNA polymerase found. Synthesis of DNA amounting to 30-80% of the added template was observed with this RNA. Actinomycin D inhibited the reaction to about 50% indicating that half of the reaction involved copying of RNA and the other half the copying of the complementary DNA into a double-stranded DNA. In order to investigate the nature of the reaction product they studied its size and its ability to hybridize specifically with 10S RNA. They were able to demonstrate that the product was completely complementary to 10S RNA and was not complementary to other RNA's found in reticulocytes and elsewhere. This RNA may be of utility in many aspects of molecular cell biology and a number of experiments have been initiated using it.

Significance to Biomedical Research and the Program of the Institute: The characterization of the enzyme that produces DNA from the tumor viruses genetic material (RNA) has the highest priority in the SVCP. It may provide much more sensitive techniques for finding cancer virus genetic information in human tumors.

Proposed Course: Continuation with slight decrease in budget.

Date Contract Initiated: May 1, 1971

Current Contract Level: $75,000

JEWISH HOSPITAL AND MEDICAL CENTER OF BROOKLYN (NCI-E-72-2034)

Title: Viral Transformation and Chromosome Abnormalities in Human Tumors.

Contractor's Project Director: Dr. Harvey Dosik

Project Officers (NCI): Dr. George Todaro
Dr. Roy Kinard

Objectives: To conduct systematic clinical, epidemiologic and cytogenetic investigations of patients and relatives of patients with chromosome abnormalities, increased risk of malignancy and those on chemotherapy for malignancy, and to supply NCI investigators with cell cultures, serum or other specimens from such patients.

Major Findings: Skin fibroblast cultures, blood cell cultures and cytogenetic analyses have been done on 70 patients and an equal number of controls. These are being studied in collaboration with Dr. Todaro to determine the extent to which genetic and chromosomal factors contribute to cellular susceptibility to transformation by both oncogenic RNA and oncogenic DNA viruses. Neoplastic and normal tissues with chromosome analyses have been supplied to SVCP on request.

Significance to Biomedical Research and the Program of the Institute: These studies are enabling the SVCP to determine, on a broader scale, the relationship between chromosome anomalies (particularly those which involve an excess of genetic material), susceptibility to cellular transformation by oncogenic agents and an increased incidence of malignancy.

Proposed Course: Continue to supply normal and neoplastic tissues from individuals with chromosome abnormalities as requested by NCI for ongoing cancer virus studies within the SVCP.

Date Contract Initiated: October 7, 1970

Current Contract Level: $84,700

OFFICE OF PROGRAM RESOURCES AND LOGISTICS

Dr. Jack Gruber, Chief, OPRL, OASDVO, Etiology Area, Chairman
Dr. David McB. Howell, OPRL, OASDVO, Etiology Area, Staff Scientist

BIOLABS, INC. (NIH 72-2068)

Title: Production of Specified Herpesviruses and the Development of Effective Production and Storage Procedures

Contractor's Project Director: Dr. Clyde R. Goodheart

Project Officers (NCI): Dr. Dharam V. Ablashi
Dr. Robert Holdenried

Objectives: A pilot study will produce, concentrate and purify EBV. The purified EBV will be checked frequently for infectivity after being preserved in various vehicles for long-term storage. Either Herpesvirus Saimiri (HVS) or rabbit lymphoma virus will be used as a model for the above objectives.

Major Findings: The contractor has recently been supplied with Herpesvirus saimiri which he has plaque purified for the production of large quantities of virus to be used in the pilot study. He has also been supplied with two continuous cell lines of owl monkey kidney to be used for HVS production. As the contract has started in the late part of December 1971, it is early to report any major results.

Significance to Biomedical Research and the Program of the Institute: Epstein-Barr virus (EBV) has been found to be a causative agent of infectious mononucleosis, a self-limiting lymphoproliferative disease similar in several respects to acute leukemia or early lymphoma. More importantly, it has been found in very close association with Burkitt's lymphoma, and it appears that all Burkitt cells contain the EBV genome integrated into the host-cell genome. EBV is therefore a prime candidate for the role of a human cancer virus, and this project will devise methods for the optimum production, concentration, and storage of this virus for use in research in the SVCP.

Proposed Course: The HVS will be first produced in large quantity to conduct pilot experiments for concentration and purification purposes. Cells carrying EBV will also be supplied for starting the production of EBV.

Date Contract Initiated: December 20, 1971

Current Annual Level: $79,090

BIONETICS RESEARCH LABORATORIES, INC. (NIH 71-2025)

Title: Investigations of Viral Carcinogenesis in Primates

Contractor's Project Director: Dr. Harvey Rabin

Project Officers (NCI): Dr. Roy Kinard
Dr. Jack Gruber
Dr. Gary Pearson

Objectives: (1) Evaluation of long-term oncogenic effects of human and animal viral inocula in primates of various species, especially newborn macaques; (2) maintenance of monkey breedin colonies and laboratories necessary for inoculation, care an monitoring of monkeys; and (3) biochemical studies of transf RNA under conditions of neoplastic transformation and studies on the significance of RNA-dependent DNA polymerase in human leukemic tissues.

Major Findings: This contractor continues to produce over 300 excellent newborn monkeys per year. This is made possible by diligent attention to reproductive physiological states of female and male breeders. Semen evaluation, artifical insemination, vaginal cytology and ovulatory drugs are used or tried as needed.

Inoculated and control infants are hand-fed and kept in modified germ-free isolators. They are removed from isolators at about 8 weeks of age and placed in filtered air cages for months or years of observation. The holding area now contains approximately 1200 animals up to 5 years old. Approximately 300 are culled every year at a rate of about 25 per month. This is necessary to make room for young animals inoculated with new or improved virus preparations.

New importance is being given to the New World species of monkeys, including squirrel, marmoset, and spider monkeys. Animals currently on study are being actively culled to reflect this change.

Special emphasis has been placed on virological studies characterizing the Mason-Pfizer monkey virus (M-PMV). Seven sublines established from chronically M-PMV-infected rhesus foreskin cultures were shown to be releasing moderately high titers of infectious M-PMV, and in addition seemed to have undergone in vitro transformation. Inoculation of cells of these sublines into newborn rhesus monkeys produced palpable masses at the sites of inoculation. Biopsies performed on these masses and on the regional lymph nodes of the same animals revealed the presence of proliferating virus characteristic of M-PMV by both electron microscopic and cell culture

analysis. Proliferating M-PMV was found in the lymph nodes of monkeys inoculated with cell-free M-PMV preparations.

Chromatographic examination of transfer RNA's (tRNA's) from control and virus-transformed rat and mouse embryo cells demonstrated differences in phenyl-alanyl-tRNA's and aspartyl-tRNA's. No differences were noted in the elution profiles of seryl-, tyrosyl-, leucyl-, asparaginyl-, or glutaminyl-tRNA.

The effects of 11 rifamycin derivatives on viral reverse transcriptase and on DNA polymerases from human normal and leukemic blood lymphocytes were evaluated. Compound 143-483, 3-formyl rifamycin SV: octyl oxime showed the greatest potency and inhibited all DNA polymerases from both viral and cellular origins.

The contractor also engaged in collaborative studies involving the oncornavirus, RD-114, from a human sarcoma, isolated by Drs. McAllister, Gardiner, and Huebner. The virus is being produced and supplied by Dr. Gilden of Flow Laboratories. Another virus, a human papovavirus associated with progressive multifocal leukoencephalopathy, is being supplied by Dr. Duard Walker for inoculation into newborn monkeys.

Significance to Biomedical Research and to the Program of the Institute: Inasmuch as tests for the biological activity of candidate human viruses will not be tested in the human species, it is imperative that another system be developed for these determinations and subsequently for the evaluation of vaccines or other measures of control. The close phylogenetic relationship of the lower primates to man justifies utilization of these animals for these purposes. Further study of altered transfer RNA and polymerase enzymes would determine their significance in neoplastic change and provide a basis for selection of therapeutic agents.

Proposed Course: The previously mentioned studies will be continued and expanded. Particular attention will be given to research on animals inoculated with candidate human cancer viruses, and investigations will be carried forward into the nature of neoplastic changes and their possible control at the cellular level. Collaborative efforts with other researchers within the SVCP will continue.

Date Contract Initiated: February 12, 1962

Current Annual Level: $2,153,850

CALIFORNIA, UNIVERSITY OF (PH43-63-13 and NCI-FS-8)

Title: Development and Evaluation of Cell Substrates for the Study of Cancer Viruses

Contractor's Project Directors: Dr. Stewart Madin
Dr. Neylan Vedros
Dr. Adeline Hackett
Dr. Walter Nelson-Rees
Dr. Helene Smith

Project Officer (NCI): Dr. James T. Duff

Objectives: The Cell Culture Laboratory (CCL) is physically located at the Naval Biomedical Research Laboratory (NBRL), in Oakland. The program of the CCL is funded by a contract (43-63-13) between the University of California and the NCI. In addition, maintenance and operating expenses generated by the CCL are repaid to NBRL by an interagency transfer of funds (FS-8) between NCI and NBRL. The research studies include the development and evaluation of cell substrates for the study of cancer viruses, development of large quantities of specific cell substrates, karotyping of cell cultures, and performing biophysical, virological, and cytogenetic research.

Major Findings: The contractor has distributed 1,072 ampoules or flasks of cell cultures to 117 recipients, primarily within the Special Virus Cancer Program, this year. The contractor's latest catalogue lists 1,254 cell substrates initiated or propagated and stored in this laboratory for distribution following antibiotic-free cultivation, characterization and assurance of species specificity, and freedom from microbial contamination.

The electron microscopic survey of cell lines for virus particles has been largely negative. However, EB virus particles were found in one cell line derived from tumor cells from a case of Hodgkin's disease. In addition, two other human cell lines derived from paracentesis fluid of a breast carcinoma and a breast adenocarcinoma showed signs of producing virus when examined by electron microscopy and DIPIC (demonstration of isotope labeled particles by isopycnic centrifugation). These developments will be pursued further.

The contractor has developed techniques for selection and cultivation of epithelial-like cells in vitro which apparently exclude fibroblastic cells.

Dimethyl-benzyl-rifampicin has been found to be extremely effective in reducing the transformation of mouse cells by MSV without affecting cell growth. In addition, this antibiotic inhibits the transformation of cells resulting from rescue of

a sarcoma genome, while leukemia virus production is not affected. These results support the idea that viral transformation of cells is independent of infectious virus production and that cellular enzyme systems other than the RNA-directed DNA polymerase of the virus are involved.

The contractor has developed a simplified assay system for leukemia virus, the NP-UCI cell line, which serves as an indicator of MLV replication and is as sensitive as the B/3T3+XC cell assay.

Clones of BALB/3T3 cells which were abortively transformed by SV40 have been grown to mass culture. These cells display normal growth characteristics, lack SV40 T antigen, and do not release SV40 upon fusion with monkey cells. However, despite the lack of any obvious manifestation of the viral genome, tests of these cells by DNA-DNA hybridization have revealed that two of the lines each contain several apparently complete copies of the SV40 genome per cell. Experiments with these "cryptic transformants" are continuing.

Chromosome monitoring of cell cultures continues for in-house production and experimentation, and in collaborative efforts, as well as for karyotype reference.

Proposed Course: Continue to develop cell reagents as substrates for human carcinogenesis; attempt to isolate and characterize viral agents from human tumor cells; continue a reference laboratory karyology of cells in culture; study oncogenic viral antigens during embryogenesis and continue basic research in the biology of tumor viruses.

Significance to Biomedical Research and the Program of the Institute: The contractor has developed an excellent tissue culture facility and is supplying cell cultures for cancer research studies to NCI investigators and SVCP contract laboratories. These studies are oriented toward a study of the fundamental biology of tumor cells, and the interaction between tumor cells and viruses of oncogenic importance.

Date Contract Initiated: October 1, 1962

Current Annual Level: $438,000

UNIVERSITY OF CALIFORNIA AT SAN DIEGO (NIH 70-2202)

Title: Development and Operation of a Breeding Colony of Domestic Cats

Contractor's Project Director: Dr. Alexis J. Kniazeff

Project Officers (NCI): Dr. Robert Holdenried
Miss Marie Purdy

Objectives: To develop and operate a permanent breeding colo of cats which will supply offspring for cancer research.

Major Findings: The operation of the colony has been, in general, more successful this year than last. The improvemen can be ascribed to better sanitation, better disease control, and a lower abortion rate among hormone-treated cats. As a result, the colony produced 136 pregnant animals, of which 114 were supplied to various SVCP investigators, the remainde being used for studies in the contractor's laboratory.

The induction of oestrus in cats at the facility is still being carried out by means of equine gonadotrophin. Limited studies have been initiated in this laboratory to adjust empirically the level of the drug to the hormonal state of the animal during different periods of the season. In addition, experiments are in progress to attempt to establish a single dose of gonadotrophin which would produce a satisfactory induction of estrus during the off-season months.

Arrangements are now being made to initiate the screening of cats, which will eventually be shipped in pregnancy to SVCP investigators, for their viral antibody spectrum. It is hoped that such information will facilitate the work of vari investigators who use these animals, particularly those involved with vaccine studies.

Significance to Biomedical Research and the Program of the Institute: This colony provides a major source of pregnant cats used in the Special Virus Cancer Program for feline leukemia-sarcoma studies. The cat remains an important animal for cancer research, as demonstrated by the fact that a candidate human cancer virus, RD-114, was first demonstrated in tumors from kittens which had been inoculated before birt with human cancer tissue.

Proposed Course: The contractor proposes to continue operatio to provide pregnant cats to laboratories within the SVCP along the previously described lines.

Date Contract Initiated: June 25, 1969

Current Annual Level: $172,700

CHICAGO PARK DISTRICT, LINCOLN PARK ZOO (PH43-65-1017)

Title: Marmoset Breeding Colony

Contractor's Project Director: Dr. Lester E. Fisher

Project Officers (NCI): Dr. Jack Gruber
Dr. Roy Kinard

Objectives: To provide marmosets in a quantity and quality sufficient for the needs of the research on tumor viruses conducted under Contract NIH-71-2032 with Rush-Presbyterian-St. Luke's Hospital.

Major Findings: There are now 53 breeding pairs in the colony. Production continues at the rate of at least 120 births per year with no problems.

Significance to Biomedical Research and the Program of the Institute: This contract is part of a program utilizing lower primates for testing selected laboratory specimens for oncogenic activity. The marmoset, a small, inexpensive primate, has been shown to be susceptible to several cancer viruses; newborn and young animals are in demand by SVCP investigators.

Proposed Course: The project will be continued to insure the availability of experimental animals of quality.

Date Contract Initiated: June 28, 1965

Current Annual Level: $24,059

UNIVERSITY OF COLORADO MEDICAL CENTER (NIH 69-2080)

Title: Collection of Pediatric Tumor Specimens

Contractor's Project Director: Dr. William E. Hathaway

Project Officer (NCI): Dr. Paul H. Levine

Objectives: To obtain tissues and serum specimens from pediatric oncology patients and suitable controls for collaborative studies with the SVCP.

Major Findings: Tissue, sera, and appropriate clinical information were sent to investigators within the SVCP from the contractor's pediatric sources. Specimens were made available from a wide variety of patients with diagnoses including leukemia, lymphoma, rhabdomyosarcoma, osteogenic sarcoma, hepatoma, neuroblastoma, and a variety of brain tumors. Two identical twins with acute leukemia and their families were evaluated for evidence of leukemia-associated antigens and skin fibroblast transformations. A unique family with a marked history of Fanconi's anemia was completely studied by chromosome analysis and skin fibroblasts were sent

for susceptibility to SV-40 transformation. In addition, specimens were available from families with multiple cases of cancer, a nursing mother with a history of Hodgkin's disease, a patient with several documented episodes of infectious mononucleosis prior to Hodgkin's disease and tissues from a patient with Jacob-Kreutzfeldt disease (a neurological degenerative disease caused by a slow virus) were provided to SVCP.

Collaborative studies have been undertaken to utilize the organization of a program on Hodgkin's disease and osteosarcoma throughout the city of Denver. Specimen collection is in progress for lymphocyte-mediated immunity studies, HLA typing, and serology. Historical data are being collected to evaluate the possible role of genetics, environmental factors, and manipulation of the immune system (particularly through tonsillectomy and appendectomy) in the etiology of Hodgkin's disease.

Significance to Biomedical Research and the Program of the Institute: This is a pediatric research contract of major importance to the SVCP, since it is a primary source of pediatric concer specimens for NCI and other SVCP researchers on the East Coast. A continuing supply of such specimens is absolutely necessary to the pursuit of the viral etiology of human cancer.

Proposed Course: Continue to collect serum and tumor specimens as in the past. Immunological investigations, using fresh lymphocytes and tissues, will be continued by the contractor. Additional personnel are being made available to collect specimens from adults with cancer, as well as children. In addition, individuals with high risk to neoplasms will be investigated for host factors which increase the possibility of developing cancer. Materials will be sent to NIH for virological and immunological studies.

Date Contract Initiated: June 18, 1969

Current Annual Level: $115,299

UNIVERSITY OF CONNECTICUT (NIH 69-52)

Title: Development and Maintenance of a Specific Pathogen Fr Flock of White Leghorn Chickens

Contractor's Project Director: Dr. Roy E. Luginbuhl

Project Officers (NCI): Dr. Robert Holdenried
Dr. Roy Kinard

Objectives: Establish and maintain a flock of chickens free of specified pathogens, including avian leukosis viruses, and to provide eggs for research use.

Major Findings: Approximately 13,000 eggs and cell cultures in addition were provided to cancer research. A few of the recipients were Drs. J. Beard, M. Green, H. Morgan, F. Deinhardt, P. Sarma, and G. S. Beaudreau. Fifteen specially characterized breeding birds were supplied to Dr. Beard's group.

There is no serologic evidence of the following organisms or diseases in the SPF flocks: Mycoplasma gallisepticum and synoviae, Salmonella pullorum, Newcastle disease, avian infectious bronchitis (Connecticut and Massachusetts strains), infectious laryngotracheitis, avian encephalomyelitis, CELO virus, and the three serotypes of RSV. There have been several birds displaying lesions characteristic of Marek's disease (MD), and MD viral antibodies have been detected in some flocks using the agar gel precipitin test. Nevertheless, one new flock consisting of 26 birds remains free of antibodies at four months of age. Several flocks have been characterized as gs antigen negative or as gs antigen positive. The gs status of these flocks' offspring can be reliably predicted, indicating a simple recessive genetic control for gs expression.

Significance to Biomedical Research and the Program of the Institute: The methods being developed indicate that eggs free of specified infectious organisms can be produced. A significant portion of the avian leukosis research in the United States is dependent on the continued availability of this highly controlled and monitored flock.

Proposed Course: Continued maintenance of the flock, with development of genetic lines of chickens characterized for the susceptibility of their embryos to leukosis virus. Reestablish flocks free of MD virus and continue work to provide genetically gs characterized birds.

Date Contract Initiated: June 18, 1962

Current Annual Level: $92,400

NEW YORK STATE VETERINARY COLLEGE AT CORNELL UNIVERSITY (NIH 70-2224)

Title: Feline Tumor Viral Diagnostic Laboratory

Contractor's Project Director: Dr. James H. Gillespie

Project Officer (NCI): Dr. James T. Duff

Objectives: To produce and evaluate cat viral reagents; to monitor cat cell cultures and other materials associated with cat tumors for indigenous cat viruses and other microorganisms.

Major Progress: During the previous year the contractor's packaged feline viral reagents have been titrated in a feline kidney cell line and the serum neutralizing titers of the packaged goat antisera to these viruses have been determined in the same kidney cell line (see October and June 1971 reports). The serologic relationships between the 14 strains of feline picornaviruses are still unclear. As previously reported there is a great degree of cross reactivity between these 14 strains and present effort is directed toward further testing to clarify these relationships.

The contractor has made available to the American Type Culture Collection bulk samples of a number of the feline viral reagents (Feline picornavirus strains FJ, 68 FIV [CFI], FRI-14, KCD, 5 FPL [Bolin] and 17 FRV) as well as the Carmichael strain of canine herpesvirus. Test samples of these agents were lyophilized by the ATCC and returned for titration. Results indicate that these viruses withstood lyophilization reasonably well and that this method of preservation might be considered for storage of these agents.

Stocks of feline herpesvirus were produced for a participating laboratory to enable them to evaluate a serologic response in leukemia infected cats. Study of the hemagglutinating properties of the feline herpesvirus (C_{27} 10 FVR) is continuing with the expectation of developing a standard hemagglutination inhibition test for the assay of antibody to this agent.

The feline tumor material monitoring phase of the contract program has been quite active during the past reporting peri The contractor has undertaken to do the monitoring for indigenous feline viruses for the NCI-participating laboratory of Dr. Vivian Larsen at Merck Sharp & Dohme. To date over 400 samples have been processed; moreover, additional samples of tumor cell cultures and related materials from a number of participating laboratories have been and are in the process of being screened for the presence of indigenous feline viruses.

Significance to Biomedical Research and the Program of the Institute: This laboratory is a major source of reagents an expertise concerning the feline tumor viruses, and makes both available to scientists within the SVCP. The contract provides a central laboratory where materials isolated from normal cats

and cats suffering from cancer can be sent to determine if they contain indigenous feline agents, as well as for viral identification.

Proposed Course: Additional characterization of feline virus reagents will be carried out; work will also be continued on the hemagglutination and hemagglutination-inhibition tests for feline herpesvirus. The laboratory will continue to monitor any material sent by SVCP researchers for the presence of indigenous or contaminant feline viruses, and will prepare stocks of feline viruses for SVCP laboratories upon request.

Date Contract Initiated: June 25, 1970

Current Annual Level: $42,000

DUKE UNIVERSITY (NIH 71-2132) DURHAM, NORTH CAROLINA

Title: Study and Production of Avian Leukosis Virus

Contractor's Project Director: Dr. Joseph W. Beard

Project Officer (NCI): Dr. Michael A. Chirigos

Objectives: The objectives of this project are: (1) to continue quantity and quality production of BAI strain A avian tumor virus; (2) to continue investigations on RNA avian leukosis viruses. The contractor projects a monthly production of 35 gms wet weight of plasma and 55 gms wet weight of tissue culture grown BAI strain A avian tumor virus. They will investigate in in vivo (chickens) and cell culture systems other avian viruses which include: sarcoma (without hematopoietic disease); erythroblastosis (and associated growths); myeloblastosis (and associated growths) and myelocytomatosis (and associated growths). Avian leukosis virus strains BAIA, R, ES4, and MC-29 will be employed in these studies. The contractor will employ a multidisciplinary approach to the studies of avian tumor viruses including: biochemistry, tissue culture, virology, electron microscopy, pathology, and immunology.

Major Findings: The major work has been the production and distribution of BAI Strain A (myeloblastosis) virus and leukemia myeloblast cells. A total of 244,929 mg. wet weight of plasma virus and 439,826 mg. wet weight of tissue culture virus has been distributed to 53 investigators. Those receiving more than 15 grams of virus are: S. Spiegelman, 78,572 mg. pv, 252,654 mg. tcv; M. Green, 1,646 mg. pv, 83,301 mg. tcv; J. Hurwitz, 17,221 mg. pv, 19,088 mg. tcv; R. Gallo, 21,380 mg. pv, 1,368 mg. tcv; P. Zamecnik, 20,547 mg. pv, 1,522 mg. tcv; D. Bolognesi, 16,774 mg. pv; K. Stromberg,

15,698 mg. pv; G. Todaro, 15,240 mg. pv.

In addition to producing large quantities of virus, the contractor has contributed to the preparation of specific products of AMV, e.g., purified gs antigen, labeled RNA, intact un-nicked RNA for end-group analysis, 62S viral RNA, etc., in response to requests from SVCP researchers.

The contractor has continued research on leukosis virus. Histologically the MC-29 strain has proven to be a most unusual leukosis agent with an especially broad spectrum of tumors not induced by any other avian tumor virus. Aside from the myeloid malignancy induced by MC-29, hepatocyte transformation and renal growths have also been observed. Of particular significance to the polymerase studies being conducted with AMV, it is interesting to note that AMV activity to induce myeloblastosis can be transmitted by virus particle-free RNA preparations. In vitro and in vivo investigations have shown that the MC-29 strain or a component of this strain passes properties of defectiveness similar to those of Bryan high titer Rous sarcoma virus.

A major portion of the contract consists of a detailed analysis of RNA tumor virus particles and their interactions with the host cell. Viral polypeptides are being purified for analysis of their amino-acid sequence and for preparation of specific antisera for analyzing virus antigens in infected cells. Preparations of AMV virus particle cores have been shown to be infectious for chick embryo cells in culture. Since AMV, however, is a non-focus forming virus, attention is being directed toward development of techniques to isolate cores from focus-forming viruses, and positive results have been obtained with the MC-29 virus.

Significance to Biomedical Research and the Program of the Institute: One of the major objectives of the SVCP is to explore fully all important animal model systems for the determination of the possible viral etiology of cancer in man. Avian tumor viruses induce a variety of diseases similar to those which occur in man (erythroblastosis, myeloblastosis, myelocytomatosis, reticuloendotheliosis, and sarcomas); the causative viruses have been isolated and the disease can be induced in vivo under controlled conditions which permit the study of the immunology, virology, biochemistry and therapy of the tumor virus complex. Moreover, BAI Strain A avian tumor virus is the only RNA C-type virus which is at present available in large enough quantities to permit exhaustive investigation into the biochemical makeup and behavior of both the virus and its components. As such, it represents an important model for the C-type viruses of higher animals and is an essential tool in the search for cancer viruses in man.

Future studies will depend upon large quantities of concentrated virus, which the contractor is uniquely in a position to supply.

Proposed Course: The contractor will continue to meet requests for virus and viral components, and will extend and expand the research outlined above.

Date Contract Initiated: April 19, 1971

Current Annual Level: $650,000

ELECTRO-NUCLEONICS LABORATORIES (NIH 71-2253)

Title: Development of Propagation Procedures, Purification and Characterization of Viruses

Contractor's Project Director: Mr. John Lemp

Project Officer (NCI): Dr. George Todaro

Objectives: To develop propagation procedures to produce high virus yields from cell cultures, and to purify, determine particle count per ml, and otherwise characterize the produced virus.

Major Findings: This contract was initiated on May 28, 1971, to provide the SVCP with a facility close to NCI where relatively large volumes of fluid containing RNA tumor viruses could be harvested from cell cultures, purified, and concentrated, and the cultures and their products studied and evaluated by electron microscopy.

The contractor has thus far cultured 38 cell lines. He has processed 1,570 liters of tissue culture fluid, from which he has obtained 423 ml. of highly concentrated tumor viruses of various kinds. These virus concentrates have been sent to researchers within the SVCP.

Significance to Biomedical Research and the Program of the Institute: The search for evidence of the viral etiology of human cancer must include studies on viruses present in cell cultures established from animal tumors as well as on those candidate human cancer viruses growing in either animal or human cell cultures. Large volumes of these well-characterized and concentrated viruses are essential for the preparation of specific antisera and for the biochemical, immunological, and epidemiological investigations necessary in cancer virus research.

Proposed Course: To continue the propagation of cell lines and the harvest of virus as directed by the Project Officer.

Date Contract Initiated: May 28, 1971

Current Annual Level: $403,000

ELECTRO-NUCLEONICS INC. (NIH 72-3249)

Title: Large-Scale Production of Oncogenic Viruses

Contractor's Project Director: Mr. John Lemp

Project Officer (NCI): Dr. Jack Gruber

Objectives: To provide research and services related to the isolation, large-scale production, concentration, and assay of oncogenic viruses of animals and potentially oncogenic viruses of humans; production and quality control will involve tissue culture, electron microscopy, immunology, and various biochemical/biophysical techniques.

Major Findings: Since this is a new contract, activated in the latter part of FY '72, there are no major findings to report.

Significance to Biomedical Research and the Program of the Institute: In order to carry out important research on the biochemistry and biophysics of oncogenic animal viruses, it is imperative that large quantities of concentrated virus be available for analysis. This contract will help meet this need with oncogenic animal viruses that have been produced under rigidly controlled conditions, and will also serve to find the best means of producing and concentrating large quantities of new candidate human cancer viruses as they are discovered.

Proposed Course: Initial production will be of Rauscher leukemia virus at a volume of 50 liters/week; eventually, production will reach a volume of 150 liters/week. Additional viruses will be produced as program needs dictate.

Date Contract Initiated:

Current Annual Level: $145,000

EMORY UNIVERSITY, YERKES PRIMATE CENTER (NIH 71-2256)

Title: Maintenance of a Colony of Irradiated, Aging Rhesus Monkeys

Contractor's Project Director: Dr. Harold McClure

Project Officer (NCI): Dr. Roy Kinard

Objectives: To determine the incidence of tumors in a unique group of irradiated, aging rhesus monkeys and to supply tissue from tumors for transplantation, tissue culture and virus isolation to SVCP collaborators.

Major Findings: A group of 72 rhesus monkeys with adults ranging in age from 12 to 18 years remain from an earlier study on the effects of irradiation. Forty-seven animals received irradiation in 1956-1958, and 16 are non-irradiated controls. The remainder are non-irradiated offspring born in the last four years.

Two animals have developed malignant tumors, an adenocarcinoma and a seminoma, respectively. Samples of these tumors have been sent to Dr. Thomas Kawakami of the University of California and to Dr. John Landon of Bionetics, Inc. Serum samples have been collected from all 72 animals in the study and shipped to Dr. Kawakami for screening of both viral antigens and antiviral antibodies.

Significance to Biomedical Research and the Program of the Institute: The SVCP conducts collaborative projects for the study of relationships between the etiologies of tumors of various primates. This project provides tumor tissues and other important specimens from aging non-human primates which have been subjected to irradiation to researchers within the SVCP. At the same time the contractor conducts a screening operation for the appearance of virus-like particles or viral antigens in the monkeys. Malignant changes in these primates may provide useful information which might be applied to humans, who are also subjected to various forms of radiation as well as to the natural aging process.

Proposed Course: The entire group of monkeys will continue to be monitored for neoplasia by physical and hematologic examinations. All tumors which develop will be evaluated by the contractor by light and electron microscopy. Specimens from these tumors will also be made available to other SVCP investigators. In addition, a breeding program is being initiated to evaluate the incidence of leukemia or other tumors in infants with aging and irradiated parents.

Date Contract Initiated: May 1, 1971

Current Annual Level: $24,000

FLOW LABORATORIES, INC. (PH43-65-1012)

Title: Maintenance of a Repository for Storage and Distribution of Reagents and Tissue Specimens

Contractor's Project Director: Mr. Jack W. Walker

Project Officer (NCI): Miss Marie E. Purdy

Objectives: To provide for the SVCP a centrally located low temperature storage and distribution center for viral reagents and tissues.

Major Findings: In 1971, the contractor made 318 shipments of viral reagents and tissues which comprised a total of 10,220 vials of material. He received 51 shipments of similar materials which comprised 25,366 vials. All incoming shipments were carefully checked for damage in transit and were catalogued before being placed in the low temperature repository.

No problems which might interfere with the efficient operation of the contractor's activities have been reported.

Significance to Biomedical Research and the Program of the Institute: An efficient research program must have readily accessible adequately characterized resource materials. The storage and shipping facilities operated under this contract enable the scientist to have access to a large inventory of special research materials without the burden of procurement, storage, inventory, and distribution.

Proposed Course: It is anticipated that the repository activities will continue to grow in the future as the Program increases in scope.

Date Contract Initiated: June 22, 1965

Current Annual Level: $115,420

FLOW LABORATORIES, INC. (NIH 71-2341) ROCKVILLE, MARYLAND

Title: Animal Holding Facility to Support Intramural Research on RNA Viruses and Autoimmune Diseases

Contractor's Project Director: Dr. William A. Knapp

Project Officer (NCI): Dr. Adi Gazdar
Dr. John W. Pearson

Objectives: The objective of this contract is to support ongoing activities in the Virus and Disease Modification Section, VBB, NCI and the Viral Pathology Section, VLLB, NCI. The nature of the above activities require supportive services which this contract provides. In general, the contractor will receive and maintain mice, rats, hamsters and other small animal species as required for the purpose of observation and experimentation during the aging process for the following research studies: (a) Development of autoimmune disease, (b) Relationship of autoimmune disease to oncogenic viruses, (c) Development of spontaneous cancers and their modification to chemo-immunotherapeutic agents, and (d) Immunologic responsiveness to immuno-enhancers and/or suppressors.

Major Findings: Since the initiation of this contract on June 15, 1971, the contractor has received 5866 mice, 1801 rats, and 4 rabbits which are being used in two projects. The first project comprises immunostimulation studies on AKR mice, to which single and multiple injections of BCG and Corynebacterium granulosum are administered over a period of time in order to prolong the time to onset of spontaneous lymphocytic leukemia. This research is at too early a stage to permit definite conclusions. In addition, the project includes the use of combination drug therapy against spontaneous leukemia in AKR mice in order to induce remission. Having succeeded in obtaining remission, the investigators are now treating the animals with single or multiple injections of BCG or Corynebacterium granulosum in order to extend the period of remission and/or obtain long term survivors of the disease.

The first project also includes studies in which rats are inoculated with various malignant cell lines, treated with drugs after a tumor appears, and then given BCG or C. granulosum if remission occurs. The cell lines include Dunning tumor cells, Nova leukemia cells, Gross leukemia cells, and Moloney sarcoma cells. These studies are still at an early stage.

Investigations are also being carried out which involve immunological studies on the C-type viruses associated with different rat tumor cell lines (R-35, WF-1, and RMTL-8). The results of immunofluorescence experiments, verified by absorption studies, indicate that the rat viruses associated with these different cell lines are antigenically identical or closely related.

The second project in this contract involves a study of oncogenic viruses and interferon inducers on autoimmune disease in NZB/WF_1 mice. This study is still at an early stage.

Significance to Biomedical Research and the Program of the Institute: The major goals of SVCP program are: (1) to detect and isolate tumor viruses, (2) the prevention and control of virus-induced malignancies in man as well as in animals associated with him. Current in-house efforts are underway to investigate the relationship of the aging process and autoimmune disease states to spontaneous and virus-induced malignancies. In addition, ongoing investigation utilizing chemo-immunotherapeutic approaches are being studied in both rat and mouse leukemia and tumor model systems. It is expected that the information resulting from this research will have considerable application in studies on similar diseases in man.

Proposed Course: It is intended to engage in inoculation, palpation, blood smears, sera collection, eye bleedings, autopsies, and harvesting and processing of tumors in mice, rats, and hamsters, and to continue maintenance of these animal colonies for long-term studies on (1) autoimmune disease in New Zealand mice, (2) development of spontaneous cancer in AKR mice and their modification by chemo-immunotherapeutic agents, and (3) immunological responsiveness to immunoenhancers, i.e. BCG, Corynebacterium granulosum, interferon stimulators, and/or immune cell transfer alone or in combination with drug therapy in various mouse and rat leukemia tumor systems.

Date Contract Initiated: June 15, 1971

Current Annual Level: $85,485

GEORGETOWN UNIVERSITY (NIH 72-3248)

Title: Supply of Blood and Tissue Specimens from Patients with Malignancies

Contractor's Project Director: Dr. Gerald Sandler

Project Officer (NCI): Dr. Paul Levine

Objectives: To collect fresh blood and tissue specimens from patients suffering from various neoplasias.

Major Findings: Since this new contract was activated in the latter part of FY '72, there are no major findings to report.

Significance to Biomedical Research and the Program of the Institute: It is vitally important that a continuing supply of specimens from patients suffering from neoplasias be available to researchers seeking the viral etiology of cancer. This contract is particularly advantageous because the contractor is located within a few miles of NCI. This

proximity will allow the formulation and alteration as necessary of particularly detailed protocols, and will also allow for the availability of very fresh specimens to SVCP researchers in the Washington area.

Proposed Course: Procurement as described.

Date Contract Initiated:

Current Annual Level: $16,517

GERMFREE LIFE RESEARCH CENTER (PH43-65-95) FT. LAUDERDALE, FLORIDA

Title: Germfree Research and Operation of a Collaborative Germfree Tumor Virus Laboratory

Contractor's Project Director: Dr. Joel Warren

Project Officer (NCI): Dr. M. A. Chirigos

Objectives: The activities under this contract are of three different types, each involving the special germfree and environmentally controlled SPF facilities and capabilities of the contractor: (1) Service type activities in general support of SVCP and intramural research requiring clean, defined animals and viral reagents; (2) Participation in collaborative research primarily of other groups, but in which germfree and/or rigid SPF environmental control are required for definitive results; and (3) Research primarily by contractor scientists, but of mutual interest to the SVCP or collaborative with other SVCP groups where back-up in other special disciplines are necessary. Specific objectives under (1) are: (a) Production of tumors in germfree avian and rodent animals by chemical carcinogens and propagation of such tumors by transplantation as a resource for studies of such tumors by other investigators. (b) Production of oncogenic viruses in germfree animals as a source of "reagent grade" virus for use in SVCP research, including that in this contractor's facility. (c) Supply of limited number of germfree animals to other investigators where feasible, as a spin-off from maintenance of the foundation colonies.

Major Findings: During the past year, numerous germfree (GF) and specific pathogen-free (SPF) animals have been sent to various researchers. Among these are: Dr. Robert Huebner, SPF Graffi hamsters; Dr. Sol Spiegelman, GF Fischer rats; Dr. William Moloney, SPF Fischer rats: Dr. Padman Sarma, GF Fischer rats; Dr. John Pearson, Fischer rats; Dr. Vernon Riley, GF BALB/c mice and GF Fischer rats; Dr. V. Saurino,

GF BALB/c mice and SPF Japanese quail; and others. Reagent grade colonies of germfree and SPF Fischer and Sprague-Dawley rats, BALB/c mice, Graffi hamsters, and Japanese quail continue to be maintained, monitored, and distributed at the request of intersted laboratories. The germfree colonies continue to be free from virus particles as determined by electron microscopy.

The contractor has found that inoculation of tobacco tar condensate into the pectoral muscle of germfree quail failed to induce neoplasia after 18 months. However, a single intramuscular injection of certain tars will accelerate and intensify the response to a subsequent inoculation of Rous sarcoma virus (RSV). The ability of tobacco tar condensate to potentiate RSV infection in quail might provide a rapid and reliable assay of potential carcinogenic activity. Tests of 20 coded samples of different tars indicate that certain preparations are more active than others in repeated assays.

Dimethylsulfoxide (DMSO) has been found to potentiate MSV infection in mice if it is incorporated into drinking water at 2% concentration. However, the same concentration of DMSO in the drinking water of rats delayed the development of transplantable leukemia in Fischer rats and markedly suppressed it in Sprague-Dawley rats.

Prolonged exposure of rat embryo fibroblast cultures to an "active" tobacco tar and subsequent inoculation of the cultures into weanling Fischer rats has given rise to tumors in three of five animals four months later. Similar cultures exposed to an "inactive" tar have not produced any symptoms to date.

Significance to Biomedical Research and the Program of the Institute: The study of many of the most important problems in the virus causation of cancer in animals requires the use of certified animal hosts free of extraneous viruses and other pathogenic microorganisms, as well as skilled technical operations within evnironmentally controlled facilities, including completely germfree isolators for some purposes.

This contract supports critical studies in such animals of interactions among viruses in dual infections (e.g., interference, enhancement, "helper" action), and cocarcinogenic activity between viruses and chemical compounds, both of which underlie the development of knowledge and technology for the detection and isolation of oncogenic viruses of man.

Future Course: To continue to develop, maintain, and distribute colonies of germfree and SPF mice, rats, and hamsters as required by SVCP projects. To continue to serve as the

nucleus colony for other contracts and intramural laboratories. To continue the production of "reagent grade" lots of the Vogt strain of RSV in germfree quail for use in research in this and other SVCP contracts; appropriate bacteriological and histopathological monitoring will be continued.

Date Contract Initiated: April 16, 1965

Current Annual Level: $110,910

HEALTH RESEARCH INC. (RPMI) (NIH 72-3247)

Title: Procurement of Leucocytes and Tissue Specimens for the SVCP

Contractor's Project Director: Dr. Joseph Sokal

Project Officer (NCI): Dr. Paul Levine

Objectives: To collect tissues and blood samples from adults suffering from neoplastic hematologic disorders, particularly Hodgkin's disease and leukemia, for use by researchers within the SVCP.

Major Findings: Since this new contract was activated in the latter part of FY '72, there are no major findings to report.

Significance to Biomedical Research and the Program of the Institute: A major goal of the SVCP is to identify, isolate, propagate, and characterize candidate human cancer viruses. Of paramount importance in the efforts to reach this goal is the continued availability of clinical specimens and histories from patients suffering from cancer. This contractor, who sees a large number of these patients annually, is in an excellent position to help meet increasing program needs for large quantities of human neoplastic specimens.

Proposed Course: Procurement as described.

Date Contract Initiated:

Current Annual Level: $15,000

HOSPITAL FOR SICK CHILDREN (PH43-65-97)

Title: Human Leukemic and Normal Tissue Collection and Preservation

Contractor's Project Director: Dr. Peter McClure

Project Officers (NCI): Dr. Paul H. Levine
Dr. Charles W. Boone

Objectives: To obtain serum and plasma specimens for a wide variety of research purposes from pediatric leukemics, relatives of such patients, and non-leukemic controls.

Major Findings: In accordance with the serum collection program, 491 samples were collected from 34 untreated acute leukemia patients, 32 "normal" controls, 31 hematological controls, 269 first degree relatives of leukemic patients, and 125 previously diagnosed leukemic patients. 548 special samples were collected from long term survivors with acute leukemia, pediatric patients with solid tumors and their first degree relatives, and a special group of normal controls. In addition to the increased number of serum samples processed, a collaborative study on leukemia in twins was continued with NCI investigators.

Significance to Biomedical Research and the Program of the Institute: As the largest pediatric hospital in North America, this contractor supplies many vital serum specimens to SVCP not readily available elsewhere. The contractor also provides an important service to Program by collecting numerous samples of tissue for uses in which the unavoidable delay in passage through customs is not critical. In addition, the contractor consistently refers patients to NCI for study whose cases are felt to be particularly relevant to the research needs of program.

Proposed Course: The contractor will continue to seek other patients of interest, and will continue to collect serial specimens from pediatric patients and family members. Virological and immunological studies on the twin families already identified will also be carried forward.

Date Contract Initiated: February 3, 1965

Current Annual Level: $15,000

HUNTINGDON RESEARCH CENTER (NIH 69-54)

Title: Development of Oncogenic Virus Diagnostic Reagents and Services

Contractor's Project Director: Dr. Roger E. Wilsnack

Project Officer (NCI): Dr. Robert Holdenried

Objectives: To develop, produce, and characterize special diagnostic reagents for use in the SVCP, primarily antisera and antisera conjugates to viruses, GS antigens, globulins of various animal species, and to T-antigens of polyoma and SV40 in tumored hamsters.

Major Findings: This year, as well as last, tumored Fischer rat MSV(M) antiserum was the most heavily and widely used of the reagents produced by the contractor. However, the contractor also continued to produce and characterize antisera against a very wide array of antigens encountered in cancer research, and added a number of antisera to his inventory during the year. These included new antisera against human immunoglobulins, porcine gamma globulins, murine leukemia virus, and feline leukemia virus. He also provided a sensitive means for the identification of group-specific (gs) antigenic activity by comparison of precipitin bands formed in a counterelectrophoresis system.

The contractor is continuing to provide antisera specific for interspecies, gs-1 type antigen by two approaches: (1) purification of immunizing antigens and (2) through utilization of the time-sequential response of the immunized host, selection of a harvest time to correspond with the greatest differential between the interspecies gs antibody titer and the titer of other antibodies (notably gs-3 antibody). Antibody titer is being measured by both counterelectrophoresis and complement fixation. Studies in progress indicate that the immune response in swine remains specific for interspecies antigens for about 30 days.

Goats, swine, rats, and rabbits are being employed as hosts for antigens used for the continued production of oncogenic virus antisera.

Significance to Biomedical Research and the Program of the Institute: The reagents and test systems developed and produced by the contract are vital tools in cancer research. The project functions in close collaboration with SVCP research projects and is felt to be of very significant usefulness to the needs of the program.

Proposed Course: To continue development, characterization, and production of antisera and serological test systems.

Date Contract Initiated: June 2, 1963

Current Annual Level: $287,045

JOHNS HOPKINS UNIVERSITY (NIH 69-2008)

Title: Maintenance of a Flock of RIF-Free Chickens

Contractor's Project Director: Dr. Frederik B. Bang

Project Officer (NCI): Dr. W. Ray Bryan

Objectives: To maintain the small "closed" flock of leukosis-free White Leghorn chickens to supply fertile eggs for use in avian tumor virus studies and to continue development of a potential continuous chicken cell line.

Major Findings: The twelfth generation of the original flock is now in egg production. Eggs are used in cell culture and avian tumor virus studies at Johns Hopkins University.

Significance to Biomedical Research and the Program of the Institute: This flock supplied the birds used by Dr. Bang in his important studies on avian tumor viruses. There is no recognized continuing need for either this flock or cell line in the SVCP.

Proposed Course: Terminate

Date Contract Initiated: March 24, 1969

Current Annual Level: $22,308

LIFE SCIENCES, INC. (PH43-68-711)

Title: Production of Germfree and Reagent Grade Specific-Pathogen-Free Animals

Contractor's Project Director: Dr. Wendall M. Farrow

Project Officers (NCI): Mr. John P. Kvedar
Dr. W. Ray Bryan

Objectives: To produce both germfree and specific-pathogen-free (SPF) animals for research. SPF animals are maintained under environmentally controlled conditions which preclude intercurrent infection by pathogenic microorganisms or infestation by parasites and are referred to as "reagent grade" hosts.

Major Findings: The contractor's supply flock of SPF leukosis-negative White Leghorn chickens, housed in a 2600-square-foot area protected by a shower lock and recirculated, filtered air, now produces about 750 fertile

eggs/week. This quantity represents a major increase in production undertaken to meet increased program requirements.

Two smaller foundation flocks (designated as LSI-C68) of pedigreed chickens are maintained in separate SPF cubicles in the maximum security area. These pedigreed chickens are continually monitored for freedom from viral and other pathogenic agents and checked for egg production. Selective breeding has produced two flocks of barrier contained, reagent grade chickens with 75-80% fertility and 85-90% hatchability. The results of these efforts are reflected in the quality and quantity of the eggs and chicks from the production flock.

A production flock of Japanese quail consisting of 235 birds continues to supply fertile eggs to SVCP users at a rate of about 1100/week. In addition, it provides a steady input of 21 to 28-day-old quail to program. Representative sampling of aged quail in this production flock indicated that these birds were freed of the usual avian pathogens. This outbred quail flock is gradually being replaced by pedigreed, isolator-derived, cubicle reared quail. Within six months pedigreed birds will compose approximately 50% of the production flock.

An inbred pedigreed foundation colony of Balb/c mice free of all laterally transmitted viruses tested for continues to be maintained. Two random bred production colonies derived therefrom continue to supply certified SPF mice to certain SVCP investigators. In addition, a foundation colony of certified SPF NIH Swiss mice is being maintained as insurance against loss to program of this valuable stock.

Significance to Biomedical Research and the Program of the Institute: This contract serves as an essential supply of embryonated eggs and day-old chicks to contract NIH 69-93, which involves studies on Marek's disease as a model for herpesvirus-associated oncogenesis. It also provides other SVCP investigators with genetically and microbiologically well-defined laboratory animals. The advantage of having such animals is that oncogenic and suspected oncogenic viruses can be administered to them with a minimal danger of interference from other contaminating, adventitious microorganisms. Therefore, research can be carried out upon animals with a known and controlled viral flora, and cell lines can be derived from these animals which share this same advantage.

Proposed Course: This service-type contract for the production of germfree and reagent grade SPF animals will be continued, with the flexibility of being reoriented as rapidly as possible to meet changing needs of SVCP activities as they occur.

Date Contract Initiated: February 8, 1968

Current Annual Level: $416,806

UNIVERSITY OF LOUISVILLE (PH43-66-902)

Title: Preparation of Simian Foamy Virus Reagents and Antisera

Contractor's Project Director: Dr. Paul B. Johnston

Project Officer (NCI): Dr. Robert Holdenried

Objectives: To prepare and test reference reagents (virus and corresponding antisera) for the simian foamy viruses, types 1-7, and foamy virus from other laboratory species.

Major Findings: The seven types of simian foamy viruses and antisera against them have been prepared, packaged, and tested for homogeneity, potency, and purity. Although primary or subcultured rabbit kidney cells remain the only satisfactory "universal host cell" for the propagation of all seven types of simian foamy viruses, a normal rat kidney (NRK) cell line, received from Dr. George Todaro of NCI, has supported excellent growth of all foamy viruses except type 4. The NRK line was subsequently used to prepare 3 liters each of types 5 and 7 foamy virus which were shipped to Dr. Maurice Green.

In tests to determine relationships between the simian foamy viruses and viruses from other laboratory animals, it was found that inoculation of PRK or BHK-21/13 cells with Mason-Pfizer monkey virus (M-PMV) did not produce syncytia after prolonged incubation under conditions optimal for detecting syncytia of all seven types of foamy viruses. Antiserum against M-PMV was devoid of neutralizing antibody against foamy virus type 5. In addition, none of the seven foamy virus antisera neutralized feline syncytial virus.

Consultation and/or foamy virus reagents are being provided to other SVCP investigators on a regular basis.

Significance to Biomedical Research and the Program of the Institute: The simian foamy virus reagents will be used in the identification of viruses and viral antibodies in primates used for cancer research. The indigenous viruses of laboratory primates pose husbandry problems, in addition to contaminating test systems and complicating the attempts to recover oncogenic virus from tissues and tissue extracts. The specific antisera may also be useful in suppressing the growth of these adventitious viruses in primate tissue cultures.

Proposed Course: The packaged foamy virus reagents now in low temperature storage will be checked for titer stability at selected time intervals. This laboratory will also assist in the detection of foamy virus contaminates on a referral basis, as well as continuing to provide foamy viruses and antisera to SVCP investigators.

Date Contract Initiated: June 13, 1966

Current Annual Level: $22,000

MELOY LABORATORIES (PH 43-66-458)

Title: Murine Mammary Tumor Virus Studies

Contractor's Project Director: Dr. John E. Verna

Project Officers (NCI): Dr. Louis R. Sibal
Dr. Ray Bryan

Objectives: To propagate, concentrate, and distribute murine mammary tumor virus (MTV) for collaborating SVCP investigators; to perform immunological and biological assays for the detection and quantitation of MTV; to develop improved methods for the propagation and detection of MTV and MTV antigens; to conduct studies on the control of neoplasia in the susceptible murine host by vaccination with inactivated virus.

Major Findings: The primary purpose of this contract is the production of quality reagents for the study of the mouse mammary tumor virus system as a model for the further examination of the human breast cancer problem. The contractor is purifying MTV from the milk of C_3H mice by the combination of rate zonal and isopycnic centrifugation. Purified virus is employed in the following ways: (a) as a source of supply to various SVCP investigators, (b) as a reagent in the HA, HAI, and ID tests, (c) employed to produce MTV antiserum in rabbits and guinea pigs, and (d) used in cell culture experiments.

Purified virus and/or viral antisera and skim C_3H mouse milk have been sent to the following investigators: Dr. R. Gilden, Dr. G. Todaro, Dr. G. Smith, Dr. W. Yoklick, Dr. A. Gazdar, Dr. A. Rabson, Dr. D. Moore, Dr. P. Blair, and others.

Some additional studies that are associated with the contract include the serological testing of human mild and human sera as well as human and mouse tumor extracts and cell culture homogenates that are received through the Project Officer or that are generated through the developmental phase of this

contract. These samples are examined for the presence of MTV antigens or antibodies. Experiments are currently in progress which attempt to demonstrate the specificity of the reactions that have detected common or cross-reacting antigens or antibodies in human milk and sera. Complement fixation assay and protein analysis have been added to the capabilities of the MTV assay laboratory and serve to supplement the present assays.

A developmental phase of the contract is concerned with the establishment of an in vitro cell culture source of MTV. Some preliminary data have been accumulated which demonstrate that MTV synthesis does occur in mammary tumor cell cultures. The presence of hydrocortisone, prolactin, and insulin results in an increased amount of virus synthesis as determined by HA and HAI. Even though marked stimulation of virus synthesis is observed in primary cultures in the presence of prolactin, hydrocortisone, and insulin, the conditions under which the positive cells can be cloned to obtain a culture with a high percentage of virus producing cells still remains unresolved. Experiments which are designed to preserve and stimulate virus synthesis in the MTV positive cells are now in progress.

Significance to Biomedical Research and the Program of the Institute: Breast cancer is a leading cause of death from cancer among women. The finding of a virus, resembling a Type B RNA oncogenic virus of mice, in the milk of a significant number of women from high-risk breast cancer families strongly suggests a possible viral etiology for this disease. A major effort of the Special Virus Cancer Program will be directed toward determining the relationship of viruses to human breast cancer. This contract was established for the purpose of obtaining correlative information on the detection, isolation, and propagation of a murine mammary tumor virus, because this is the only available animal model system in which approaches to the study of viruses as a cause of breast cancer in many may be developed.

Proposed Course: Purified MTV, viral reagents, and mouse milk will continue to be supplied as needed by SVCP investigators. In addition, the biological and immunological methods developed in this laboratory will be used in systematic studies to develop further the mouse MTV system as a laboratory model for breast cancer virus studies in man. Greater emphasis will be placed on propagating this virus in tissue culture and on in vivo infection of cells in cultures. It is anticipated that the information gained from these studies will be applied to human breast cancer studies.

Date Contract Initiated: December 30, 1965

Current Annual Level: $688,212

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NIH-71-2116)

Title: Acquisition of Human Materials for Use in the Search for Transmissible Agents in Human Tumors

Contractor's Project Director: Dr. Yashar Hirshaut

Project Officer (NCI): Dr. Jack Gruber

Objectives: To gather sera and tissues from patients with tumors to be used in the search for tumor-specific antigens and human oncogenic viruses.

Major Findings: The tumor procurement program at Memorial Hospital for Cancer and Allied Diseases was established on March 1, 1971 under contract with the Special Virus Cancer Program. It operates as an independent administrative unit called the Tumor Procurement Center, but is closely affiliated with the Clinical Immunology Service of the Department of Medicine, Memorial Hospital. In the first 3 months of its existence, the administrative groundwork for the program was laid, a competent staff was hired, and arrangements were made for efficient tumor collection and data processing. Operations began on June 1, 1971 and since that time, nearly 5000 serum specimens have been collected, 591 surgical cases have been screened, 164 tumor samples have been gathered, and 69 effusions obtained. Numerous samples have been distributed to investigators in the SVCP, including Drs. Hidesaburo Hanafusa, David Yohn, Sol Spiegelman, Robert Gallo, Harry Eagle, and Anthony Girardi.

Significance to Biomedical Research and the Program of the Institute: In the last ten years, rapid progress has been made in the study of oncogenic animal viruses. Unfortunately, human studies have frequently been limited by the lack of suitable materials to be used in virus isolation and detection attempts. The procurement program at Memorial Hospital for Cancer and Allied Diseases in New York City provides cooperating investigators with sufficient numbers of specimens from tumor-bearing patients to permit them to undertake intensive studies of the possible viral etiology of human cancer.

Proposed Course: In the coming year, a modest expansion of the program is foreseen to take greater advantage of the extensive resources of Memorial Hospital. With the experience gained in the first contract year, it should be possible to service additional investigators. The close collaborative

relationship between the procurement program and participating investigators has assured that the materials provided are optimal for the needs of each laboratory. It is hoped that these associations will be strengthened in the future.

Date Contract Initiated: March 1, 1971

Current Annual Level: $95,000

UNIVERSITY OF MICHIGAN (PH43-65-639)

Title: Collection of Leukemia-Lymphoma Specimens

Contractor's Project Director: Dr. Chris J. D. Zarafonetis

Project Officers (NCI): Dr. Paul H. Levine
Dr. Ernest J. Plata

Objectives: To collect and distribute specimens and information from patients with leukemia or lymphoma.

Major Findings: The major efforts of the contractor have been divided between procurement of specimens, evaluation of special clinical situations, and computerization of the Simpson Memorial Institute Serum Bank. The first phase of the computerization program, which was undertaken to provide immediate and accurate information on the location and availability of specimens in the Serum Bank, was completed. The core data for 25,071 specimens have been keypunched and a program which interdigitates with PAC has evolved.

Over 4,000 serum specimens have been collected during the past year, along with various tissues from patients with breast cancer, American Burkitt's lymphoma, leukemia, Hodgkin's disease, reticulum cell sarcomas, lymphosarcomas, and malignant lymphomas. Recipients of these samples have included Drs. Spiegelman, Terasaki, Herberman, Levine, Gallo, Aaronson, Todaro, and numerous others within the SVCP.

Significance to Biomedical Research and the Program of the Institute: Availability of clinical specimens and pertinent information on the cases is paramount in the achievement of a major goal of the SVCP, i.e., to identify, rescue, characterize, and propagate a candidate human cancer virus. Large volumes of leukemic cells are necessary for the biochemical characterization of the polymerases present in these cells, and a large number of tissues will be necessary to keep up with the biochemical demand. In addition to helping meet this need, the contractor is also collecting a large number

of sera for the SVCP serum bank and has been able to meet new requests for a variety of specimens for SVCP investigators.

Proposed Course: Continuation as described.

Date Contract Initiated: June 21, 1965

Current Annual Level: $98,098

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-66-914)

Title: Establish and Operate a BALB/c Mouse Colony

Contractor's Project Director: Mr. Wilbur Athey

Project Officer (NCI): Mr. Samuel M. Poiley

Objectives: To provide BALB/c mice for laboratory investigations supported by the SVCP, primarily for virus bioassays on Contract 43-67-697.

Major Findings: The contractor has provided the maximum numbers of mice that can be produced in 2,500 cages. All regulations of The Institute for Laboratory Animal Resources are followed carefully. Requests for animals based on age, sex, weight, suckling litters, or breeders, etc. have been consistently met.

Production under this colony has been increased to fully utilize the capabilities of half an animal building (1050 square feet) at Walkersville, Maryland. Production during the next year should yield 60,000 to 65,000 weanling mice plus the necessary pregnants for the program. This maximum yield will be possible only if the majority of mice are used at weanling age. Significant requirements for older aged mice will reduce the total number produced.

The results from the installation of a metering dispenser to acidify the water to a pH of 2.5 have not been conclusive but should show positive results during the next contract year. The serologic testing for a battery of murine viruses is scheduled on a quarterly basis as directed. At the last regular test, all samples were serologically negative to all viruses tested.

Significance to Biomedical Research and the Program of the Institute: The murine tumor viruses are being extensively studied as models for human cancer viruses. The availability of high quality BALB/c mice is important for assay of these viruses as well as for other studies in viral oncogenesis.

Proposed Course: Mouse production will be continued at the current level.

Date Contract Initiated: June 16, 1966

Current Annual Level: $64,000

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-67-700)

Title: Development of Laboratory Animal Virus Diagnostic Reagents and Services

Contractor's Project Director: Dr. John C. Parker

Project Officers: Dr. Robert Holdenried (NCI)
Dr. Wallace P. Rowe (NIAID)

Objectives: To develop reagents and tests for the detection of murine and other laboratory rodent and cat viruses; to apply these and other tools in the determination of the importance of the indigenous viruses in experimental systems; to study means for elimination of viruses from laboratory animal populations. An additional collaborative study to assist in the characterization of the gene-dependent expression of murine leukemia was initiated in August 1971 and continues.

Major Findings: This contract project provides to the NCI a heavily used, highly skilled murine virus diagnostic laboratory. In the year ending September 30, 1971, 5,467 serological specimens were received and 52,580 tests performed. In addition, indirect tests (mouse antibody production) were performed on 116 specimens. The laboratory has the capability of performing serological test detecting infection with 32 known viruses or closely related groups of viruses.

To assist with the viral definition of cats to be used in cancer research, rapid diagnosis tests for 17 cat viruses have been developed and assayed for sensitivity. These serological tests involve either complement fixation or hemagglutination-inhibition procedures. Microneutralization assays were also developed for each of the cat viruses. These test systems are applicable to identification viral isolates or antibody.

Lymphocytic choriomeningitis (LCM) virus, which may cause serious disease in humans, continues to be of concern to research laboratory personnel using rodents. Collaboration studies revealed a significant incidence of infection in wild house mouse populations in Southern California where these mice are being utilized in a contract cancer research project.

A high incidence of LCM virus infection was also found in a commercial laboratory mouse production colony. The contract personnel assisted the producer in determining the extent of infection, preparing serological diagnostic reagents for use in another laboratory and in alerting mouse-using laboratories about the potential health hazard to their laboratory personnel.

Virus diagnostic reagents were prepared and certified for use on the in-house serological service program and research studies, for both the American Type Culture Collection and the World Health Organization virus reagent program, and for distribution on SVCP's request.

A natural history study of the mouse thymic virus, a herpes-virus, was initiated. With the development of complement fixation and fluorescent tests for this virus a major impediment to progress was overcome. The virus was detected in one of three mouse colonies examined to date. The effect of infection on immune functions particularly in relation to leukemogenesis is being investigated.

The preparation of monotypic homologous antisera in cats to 6 cat viruses was completed. The results of an investigation on the relationship of the cat picornaviruses will be presented by Dr. Parker at a WHO meeting on viral nomenclature.

Significance to Biomedical Research and the Program of the Institute: The virus diagnostic capabilities provide the NCI with the ability to monitor laboratory rodent and cat colonies and laboratory animal-produced viral reagents and tumors which have resulted in the production of highly characterized systems for cancer research. This contract provides assistance and guidance of particular importance for the detection of LCM in rodent systems. LCM virus, in addition to being infectious for humans, is difficult to detect. Significant contributions are being made to the knowledge of the natural history of several indigenous viruses of laboratory animals.

Proposed Course: Continue the rodent virus serodiagnostic service and develop a similar service for cat viruses. Improve the sensitivity and reliability of the tests. Apply the information developed to reduce and control viral infections in laboratory animal colonies and materials derived from animals.

Date Contract Initiated: April 10, 1961

Current Annual Level: $450,000

MONTREAL CHILDREN'S HOSPITAL (PH43-65-1020)

Title: Procurement of Normal and Leukemic Sera from Children

Contractor's Project Director: Dr. Ronald L. Denton

Project Officer (NCI): Dr. Paul H. Levine

Objectives: To obtain sera from a variety of pediatric oncology patients, family members, and controls for virologic study; to identify special cases for more extended workup.

Major Findings: Serum procurement during the last year included collections from 41 new cases of acute leukemia, solid tumor, 58 family members and 22 controls. 46 followup samples were obtained from previously studied patients. Among the 92 other specimens collected, special attention was given to a new American Burkitt's lymphoma patient, two patients with osteogenic sarcoma, a patient with malignant histiocytoses, and one with a congenital lesion (Birdman syndrome) associated with acute leukemia. In addition to the 1243 vials of serum and plasma that were transferred to the Flow Laboratory Serum Bank, the majority of the specimens collected by the contractor were sent to Dr. R. Gallo, Dr. S. Spiegelman, Meloy Laboratories, and Bionetics Research Laboratiries.

Significance to Biomedical Research and the Program of the Institute: This contract is one of the program's primary sources of serum from leukemic children and from suitable, normal controls. The increasing need within the SVCP for samples of this kind makes it essential that the supply be continued to satisfy research requirements.

Proposed Course: Continue to collect serum specimens, especially from selected leukemia patients, for identification of host factors associated with long-term survival.

Date Contract Initiated: September 24, 1965

Current Annual Level: $30,000

UNIVERSITY OF PADUA (PH43-68-1389)

Title: Collection of Human Tissue Specimens

Contractor's Project Director: Professor Giovanni Dogo

Project Officer (NCI): Dr. Robert H. Depue, Jr.
Dr. Charles W. Boone

Objectives: To establish fibroblast cultures from skin biopsies taken from inbred and isolated human donors and to provide these cultures to NCI for use in research programs.

Major Findings: Skin biopsies have been obtained from inbred and isolated populations in the Dolomite Mountains in Italy and have been grown in tissue culture. A sample of each culture has been sent to NCI; in addition, blood samples have been procured from each donor, and family trees going back to the fifth or sixth generation have been established for each individual. The contractor is presently using the geneological information to identify subjects belonging to family groups having a high incidence of neoplasia.

Significance to Biomedical Research and the Program of the Institute: The cell lines established from these skin biopsies will be used in a project to detect human oncogenic viruses in vitro and to determine the significance of the transformation test to oncogenesis.

Proposed Course: To collect and culture human skin biopsies as previously, along with genealogies.

Date Contract Initiated: October 27, 1964

Current Annual Level: $8,000

CHARLES PFIZER AND COMPANY, INC., (NIH-70-2080)

Title: Tumor Virus Research

Contractor's Project Directors: Dr. J. J. Oleson
Dr. Sami Mayyasi

Project Officers (NCI): Dr. Jack Gruber
Dr. W. Ray Bryan
Dr. Roy F. Kinard

Objectives: Provide research and services related to the isolation, production, purification, assay, and control of tumor viruses, including electron microscopy, tissue culture, and immunology applied to the study of animal and potentially oncogenic human viruses.

Major Findings: Recent advances have markedly increased the requirements of the SVCP laboratories for larger quantities and varieties of high grade, specifically prepared virus materials for biochemical, biological and immunological studies. New C-type viruses, of great interest and priority to the program, must be made in sufficient quantities for

in-depth studies. The contractor has responded by increasing production levels 30% to 600L/week, modifying a laboratory into a high containment production/research facility, purchasing additional centrifuges, hoods and other equipment and transferring personnel to this contract.

A total of 6,205 liters of fluids containing virus was produced these past 16 weeks of which 57% was mammary viruses and 32% was Rauscher leukemia virus.

The RD-114B sarcoma virus cell line, received late in December, has grown well and currently is being produced at 45 liters/ week. The contractor plans future scale-up to 100 liters pending installation of new centrifugation apparatus for further purification processing. Preliminary virus growth and characterization studies have shown that several different human cell lines are susceptible to infection with this virus. These results encourage attempts to develop an assay for tissue culture infectivity.

Important new information and technology have been developed with the R-35 mammary tumor virus, concerning stability, purification, cell transformation, tumor cell and virus morphology, antigenic characterization, bioassay systems and biochemical characterization.

Comparisons of the reverse transcriptase (RDDP) activities and high molecular weight RNA content in virus concentrates after varied growth, harvest, and purification conditions now guide attempts to make high quality M-PM and Rauscher viruses for biochemical experiments. With some cell culture lines the RDDP content of virus produced has been shown to be a function of the culture transfer history.

Two new simian lymphoma and sarcoma viruses have been received and the contractor plans to produce limited amounts of each.

Substantial improvements in biohazard control have been achieved with the introduction of new operational procedures, equipment and personnel indoctrination.

Electron microscope services were provided for SVCP investigators and also supported the contractor's activities.

Significance to Biomedical Research and the Program of the Institute: Since its inception, this contract has provided support to individual investigators thereby making possible research which could not otherwise have been undertaken. The research conducted in the contractor's laboratories has largely been directed to improving the quality of material support to different research activities.

Proposed Course: The production of virus and cell materials in support of pertinent research will continue.

Date Contract Initiated: November 6, 1961

Current Annual Level: $1,685,821

ST. JOSEPH'S HOSPITAL (NIH 69-2074)

Title: Study of Human Sarcomas and Their Possible Viral Etiology.

Contractor's Project Director: Dr. Jeno E. Szakacs

Project Officers (NCI): Dr. Albert J. Dalton
Dr. Roy F. Kinard

Objectives: To find and supply fresh human sarcomas or other tumors which contain EM evidence of virus particles, and to attempt to establish cell cultures from some of these tumors.

Major Findings: Continuous tissue cultures established from human sarcomas collected from Tampa Bay area hospitals are being surveyed for virus particles by electron microscopy (EM). Of the eighty sarcomas collected during the year, forty-four cultures are now available for study which range from first to twentieth passage cultures. Each second passage is surveyed by EM.

During the latter part of the year, experiments were undertaken to induce chemically the appearance of virus particles in some of these cultures. At least one of the cultures thus treated has yielded virus particles, identification of which is currently underway.

Methods of tissue culture in the contractor's laboratory have been refined in order to permit the preferential cultivation of well-characterized tumor cells. Cloning and other new methods of handling the cultures were applied to this problem; in addition, immunofluorescent studies of the cultures were extended further.

A total of twenty tissue cultures derived from human sarcomas and 265 serum samples were shipped to NCI during the year. Serum collection, both for the sarcoma project and for Serum Bank projects, is continuing.

Significance to Biomedical Research and the Program of the Institute: This is an important project concerned with the search for viruses in human tumors. Extensive and careful

examination, by electron microscopy, of a large number of human tumors and cell cultures established from these tumors is essential in determining the viral etiology of cancer.

Proposed Course: All sarcoma cultures in the contractor's possession will be placed under chemical induction, and isolation and characterization will be attempted on all virus particles so obtained. The collection of sarcomas, case histories, and sera will continue. As volume increases, tumor tissues will be made increasingly available to SVCP investigators.

Date Contract Initiated: June 24, 1969

Current Annual Level: $108,315

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NIH 69-2011)

Title: Housing and Maintenance of a Chimpanzee Colony

Contractor's Project Director: Dr. Seymour S. Kalter

Project Officer (NCI): Dr. Roy F. Kinard

Objectives: To supply young chimpanzees to SVCP investigators.

Major Findings: The animals maintained under this contract have been in generally good health during the year despite the occurrence of two deaths and several individual illnesses. Five infants (three males, two females) were born during this period, one of which, a female, was sent to Baylor Medical School along with an infant male and an infant female born late in 1970.

The chimpanzee colony now comprises the following animals: six breeding age females, one breeding age male, one juvenile male, two juvenile females, three infant males, and two infant females, for a total of 15 animals.

Significance to Biomedical Research and the Program of the Institute: The chimpanzee now appears to be the laboratory animal most similar to humans, biochemically and immunologically. Newborn chimpanzees are particularly useful in determining susceptibility to suspected human cancer viruses because their resistance to virus infection is very low. This is the only source of newborn chimpanzees for the SVCP.

Proposed Course: The chimpanzee colony will be maintained as before and newborn animals will continue to be supplied to investigators within the SVCP.

Date Contract Initiated: April 25, 1969

Current Annual Level: $25,000

UNIVERSITY LABORATORIES, INC. (PH43-66-1133)

Title: Production of Oncogenic Viruses and Antisera

Contractor's Project Director: Dr. Eugene H. Bernstein

Project Officers (NCI): Dr. Robert Holdenried
Dr. Robert Bassin
Mr. Gary Armstrong

Objectives: Production of leukemia and sarcoma viruses and antisera.

Major Findings: Within the year immediate SVCP needs for MSV(M), harvested from BALB/c mouse tumors, and the Moloney leukemia virus (I.C. strain derivation), harvested from BALB/c mouse spleens, were met and production was suspended. These viruses, in storage at the Flow Laboratories repository, are issued upon request to research projects. The production of mouse antiserum to MLV was also completed. This serum has a highly specific neutralizing titer for MLV. The production of Rauscher leukemia virus, which is harvested from the plasma of infected mice, has been initiated and is proceeding at the rate of about 400 ml. per month. Virus particle count by electron microscopy (EM) is 10^7 particles/ml., but assay of the virus on S+L- cells yields a titer of 10^3-10^4 focus-forming units/ml.

Cat leukemia virus (F-422 of Rickard) production was initiated in a continuous line of thymus cells and continues at the rate of about 4,500 ml. of culture fluid per month. Examination of the fluid by EM reveals a particle count of about 10^{10} particles/ml.

Several avian viruses are being produced in cell cultures. Rous sarcoma virus (Prague strain) is produced in the largest volume, about 25 liters of culture fluid per week. After being pelleted and resuspended, the virus is issued at 100X its original concentration. Production of Rous-associated virus 7 (RAV-7) terminated in December and RAV-2 is now being produced in its place. The production rate is about 2 liters per week.

Significance to Biomedical Research and the Program of the Institute: The supply of highly standardized oncogenic viruses and antisera produced by this contractor has been

extensively used by SVCP researchers and is essential to the continuation of many important research projects presently being carried out in the program.

Proposed Course: The contractor has largely shifted from producing virus in animals to cell culture propagation. Production of needed strains of oncogenic viruses and their antisera will continue in volumes necessary to meet SVCP research needs.

Date Contract Initiated: June 4, 1962

Current Annual Level: $322,140

WOLF RESEARCH AND DEVELOPMENT CORP. (NIH 71-2270)

Title: Computer Services

Contractor's Project Director: Dr. William Wells

Project Officers (NCI): Dr. Deward E. Waggoner
Mr. Theodore Weiss

Objectives: The major objective of this contract is the implementation of a computerized central inventory system for the various resources of the SVCP. The system now being installed embraces a number of institutional repository subsystems contributing to the central base in the Program Analysis and Communications Office (PAC). Each computerized subsystem is used at a given resource repository to inventory the items produced or stored at that location. Since the types of resources at the various resource banks differ in kind, the subsystems all differ to some extent. They are, however, all designed to contribute compatible information to the central system in PAC, thus forming the base for a program-wide central inventory and control.

The resource bank subsystems are also used at storage sites for controlling materials in functions unique to each institution, and this contributes to the detailed differences. Some of these secondary functions include catalog production, local inventory, and retrieval of clinical information.

Major Progress: A thorough systems analysis, documentation, and integration of all ongoing computer data processing of the serum bank operations in PAC was compiled and reproduced in a single manual (PAC I). In addition to its use as a compact operating manual, this served as a security measure, insuring future continuity of operations with the possibility of personnel change in a complex data milieu.

The serum bank inventory system was then redesigned to include all other human specimens (especially tissue and cell lines). It was further expanded to include a wide array of clinical, laboratory, and demographic information on specimen donors, parameters expected to be useful in the selection of specimens and in interpreting results of virus laboratory testing. Extensive documentation of the new and expanded system (PAC II) was reproduced as an operating manual. While the compact computer systems therein will have greatest use in managing the Washington area specimen banks, they are designed to adapt to any institution contributing to the SVCP human resources. Compatibility of information codes and operating format between SVCP Bethesda and cooperating institutions is an integral principle of PAC II.

Wolf Research and Development has completed, in collaboration with SVCP personnel, the following work in repository subsystems:

1. University of Michigan. Management studies were conducted for operation of the serum bank at Simpson Memorial Institute. Systems were designed, personnel trained, and descriptive data on 26,000 human specimens added to the PAC serum bank inventory.

2. Naval Biomedical Research Laboratory. Systems were studied, data forms prepared, and a proposal for automation of the cell culture repository submitted.

3. M. D. Anderson Hospital. Conversion of the data concerning the specimen repository to the new PAC II central inventory format is underway.

Significance to Biomedical Research and the Program of the Institute: The necessary expansion of the inventory of viruses, sera, tissue cultures, human specimens, and other materials used in cancer research makes it vital that close control be exercised over these resources. Computerization of the inventory will eventually make it possible for the Office of Resources and Logistics to rapidly obtain information necessary to determine availability, location, quantity, etc. of all resources within its jurisdiction, thereby permitting rapid response to the needs of the program while avoiding resource excesses or shortages.

Proposed Course: The contractor will extend and refine the Central Inventory System. Efforts will include the implementation of the Query capability and the development of a program to select control specimens matched to the characteristics of selected study specimens. Work on subsystems will include: addition of further data from M.D. Anderson to the

NCI data base; proposal and implementation of a system for Memorial Hospital (N.Y.); addition of data on reagent production from Huntingdon Research Labs.; and integration of the NBRL system into the NCI system when funded.

Date Contract Initiated: May 3, 1971

Current Annual Level: $127,300

SOLID TUMOR VIRUS PROGRAM SEGMENT

Dr. Robert J. Huebner, VCB, Etiology Area, Chairman
Dr. James T. Duff, VCB, Etiology Area, Vice Chairman

CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH
(PH43-NIH-NCI-E-68-997)

Title: Role of Oncogenic Viruses in the Causation of Cancer in Man and his Domestic Animals

Contractor's Project Director: Dr. Edwin H. Lennette

Project Officer (NCI): Dr. James T. Duff

Objectives: To apply the newer knowledge of the nature of RNA viruses to the study of neoplasms of animals and man.

Major Findings: (1) Viral genome rescue experiments: Several approaches are being explored in attempts to rescue infectious Type C viruses from nonviral producing cell lines which have been established in this laboratory from tumors of cats, dogs, and humans. These include treatment of the cultures with mitomycin C, BrdU, IdU, x-irradiation, super-infection with infectious Type C viruses, co-cultivation with normal "susceptible" cells, or combinations of these procedures. To date the results of these experiments have been negative. (2) Experiments with anti-thymocyte serum (ATS) treated mice: ATS treated Swiss mice have produced recognizable adenocarcinomatous structures when minced tumor preparations from two of four human colon carcinomas were transplanted within two days after surgery. No tumors when third passage in vitro cultured cells from the same tumors were used in the ATS treated mice. C57/Leaden mice have been bred and have proven susceptible to HeLa cell tumor formation when treated with ATS. (3) Primate tumor induction experiments (in collaboration with Dr. Melnick at Baylor University): Baboon testis cells have been transformed in vitro by both the ST and GA strains of feline sarcoma virus (FeSV). Following transformation by either virus, the cells became sarcomagenic for ATS treated mice. These cells have been injected into the original cell donor baboons, and the animals are under observation for tumor development and anti-FeSV antibody production. (4) Feline gs-1 antigen studies: Feline gs-1 antigen purified by electro-focusing was inoculated into two guinea pigs to produce antiserum. Both guinea pigs responded with high CF titers and also showed only one precipitin line in immunodiffusion tests using crude ether-treated FeLV as antigen. Low titered reactions against normal cat cell antigen and fetal bovine serum were removed by absorbing with normal cat cells grown

in cell culture and by utilizing insolubilized fetal bovine serum which had been cross-linked with glutaraldehyde as an immunoabsorbent. (5) Indirect fluorescent antibody studies: Preliminary work has begun on attempts to localize feline gs-1 antigen in cells utilizing the indirect fluorescent antibody technique. The cells first used were both dog and cat cell lines which were chronically infected with FeLV and their uninfected counterpart as controls. In every case tested, so far, both guinea pig sera described above showed bright specific staining in the cytoplasm of only the cells infected with the virus, the uninfected controls remaining negative. (6) Virus in RD 114 cell line: The indirect ferritin-labeled antibody technique was applied to RD 114 cells to determine if the virus in RD 114 cells shared a common surface antigen with the feline leukemia-sarcoma viruses. The application of XD25M serum (antibodies to feline leukemia-sarcoma virus antigens) resulted in ferritin-tagging of the viral particles in the feline leukemia virus infected RD cell lines but did not show tagging of the viral particles in the RD 114 cell lines. (7) Type C virus on erythrocytes: The sequence of events in the development of feline Type C virus on the plasma membrane of erythrocytes has been elucidated in electron micrographs. Particles were observed in various stages of development, indicating that an erythrocyte can retain the necessary information for viral replication. (8) Feline syncytium-forming virus studies: Experiments have been completed on the growth of feline syncytium-forming virus. New infectious virus is produced by 20 hours post infection (PI) while new virus antigen can be detected at 12 hours PI using the fluorescent antibody technique. Syncytium began forming between 12 and 14 hours PI. (9) Focus-forming assay of feline sarcoma virus on beagle embryo cells (NBE3): Both the ST and GA strains of FSV can be grown and assayed on cultures of a normal beagle embryo cell (NBE3). The most sensitive titrations have been obtained on cells between the 30th and 50th passages. The FSV pools produced contain an excess of nontransforming (helper) virus as was shown in cell cultures inoculated with virus dilutions beyond the focus-forming endpoint (usually 10^{-3}).

Significance to Biomedical Research and the Program of the Institute: These studies are a part of a comprehensive field program on the etiology of cancer in man and his domestic animals being carried out as a collaborative effort between the California State Department of Public Health (NIH-NCI-E-69-87), the University of California (Naval Biomedical Research Laboratories, PH43-NIH-NCI-E-63-13), the University of Southern California and Los Angeles Children's Hospital (PH43-NIH-NCI-E-68-1030), Stanford University (NIH-NCI-E-69-2053), and other contract programs, including

in-house projects under VCB. To a large extent, the success of these programs depends on the acquisition of fresh human and animal (pet) cancer materials, cell lines derived from them, and sera from persons associated with pets with cancer. This group has responsibility for developing diagnostic techniques for feline Type C viruses and applying them in studies of the natural history of the cat virus for the purpose of applying what is learned to the natural history and etiology of human cancer. A major new objective will be to carry out fluorescent antibody studies of human tumor cells to study the possible interactions of human tumors with the RD 114 virus. Also, the development of methods of replicating human tumor cells in animals and of detecting Type C virus expression in them.

Proposed Course: (1) Continue to obtain cats and dogs with malignancies from local veterinarians in order to study their immunological relationship. (2) Continue to establish cell cultures from human tumors to study their tumorigenicity in ATS treated mice for the purpose of (a) verifying that they are tumor cell lines instead of the usual fibroblasts and (b) trying to "switch-on" human Type C virus by in vivo passage similar to the switch-on which occurred in RD cells on passage through a cat.

Date Contract Initiated: June 24, 1968

Current Contract Level: $227,715

CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH (NIH-NCI-E-69-87)

Title: Human-feline Cancer Household Study

Contractor's Project Director: Dr. Robert Schneider

Project Officer (NCI): Dr. James T. Duff

Objectives: To determine if a significant etiological association exists between human and feline cancer using a retrospective study of households in which a malignant cancer has been diagnosed in a cat, and concurrently to look for and test the significance of apparent horizontal transmission of lymphoma virus from cat to cat.

Major Findings: The field and coding phases of the Animal Population Survey for Alameda and Contra Costa Counties have been completed. Of 31,165 valid households, 26,940, or 86.4%, were interviewed. Initial estimates indicate dog and cat population of approximately 250,000 and 200,000, respectively, in the two counties. A little over 40% of all households own either a dog or a cat or both. The human

population of the area is approximately 1,500,000.

No evidence for transmission of feline oncogenic agents to man or dogs was found, and data collected on 221 feline malignant lymphoma cases and matched control cats did not support the concept that horizontal transmission of infectious FeLV from cat to cat is an important mode of transmission. However, this would not exclude (a) occasional horizontal transmission and (b) transmission of infectious cat lymphoma virus in cats genetically resistant to lymphomatous or cancerous manifestations.

Significance to Biomedical Research and the Program of the Institute: The Animal Neoplasm Registry (ANR) of the California State Department of Public Health has been functioning since July, 1963. All cases are histopathologically confirmed and information is kept about the animal and the owner. This epidemiological study is important for determining the possible effects of exposure to cat leukemia and sarcoma viruses on human cancer and the importance of horizontal spread of virus from cat to cat and from cat to dog in maintaining the natural history of the feline viruses.

Proposed Course: The epidemiological survey will continue to completion.

Date Contract Initiated: June 19, 1969

Current Contract Level: $35,000

CALIFORNIA SCHOOL OF MEDICINE, UNIVERSITY OF (NIH-NCI-E-71-2147)

Title: RNA Directed DNA Polymerase and the Replication of Rous Sarcoma Virus

Contractor's Project Directors: Dr. J. Michael Bishop
Dr. Warren Levinson
Dr. Leon Levintow

Project Officer (NCI): Dr. Bernard Talbot

Objectives: Conduct investigations on the molecular biology of the avian and other RNA tumor viruses, particularly (a) the virion-associated DNA polymerase and (b) the virus-specific DNA and RNA in normal and transformed cells.

Major Findings: (1) Purified preparations of Rous sarcoma virus have been found to contain small amounts of double-stranded DNA; this DNA cannot be hybridized to viral RNA, but will reanneal completely with the DNA of avian cells. It thus appears to be derived from the DNA of the avian host

cell and is probably devoid of any function in the life cycle of the virus.

(2) Improved assays for virus-specific DNA and RNA in normal and infected cells have been developed. Both assays utilize the exceptional senstivity and specificity of single strand-specific nucleases from Neurospora crassa and Aspergillus oryzae. (a) The amount of virus-specific DNA present in cells is independent of the presence or absence of viral gs antigen, independent of infection with and transformation by oncornavirus, and independent of autosomal dominant traits determining a high incidence of virus-associated mammary carcinoma. For example, there was no genotypic difference detectable in the amount of MTV-specific DNA between the GR (high incidence of MTV and mammary cancer) strain and the C57BL/6 (low incidence) strain. This suggests that the inherited difference between the two strains resides in regulatory genes which govern the expression of viral information. Also, there were a similar number of multiple DNA copies of avian tumor virus genes found in normal or transformed chicken cells. These results suggest that all chicken cells contain a considerable amount of avian tumor virus genetic information and that the information may then be expressed in response to various factors such as inheritance of regulatory genes or stimulation by physical, chemical, or biological agents. (b) Virus-specific RNA has been detected in the nucleus and cytoplasm of infected cells, and the size of this RNA has been determined. Virus-specific messenger RNA has been isolated. A surprising finding is the presence of MTV specific RNA in the normal lactating breast of the C57 (low incidence) mouse. It is now feasible to perform detailed studies on the kinetics, chronology and mechanism of replication of viral RNA.

(3) The reverse transcriptase of Rous sarcoma virus has been purified 1,000-fold (2-4 molecules/virion) and found to consist of a maximum of two polypeptides with molecular weights 105,000 and 68,000. The purified enzyme closely resembles the polymerase activity of detergent-disrupted virions in both template response and nature of the DNA synthesized. The product DNA is small, and much of it is preferentially transcribed from a limited portion of the viral genome.

(4) DNA synthesis by the enzyme with 70S RNA as template is initiated on the 3' terminus of a polyribonucleotide. This primer molecule has been isolated and provisionally identified as 4S RNA known to be hydrogen-bonded to 70S RNA. Among the DNA polymerases tested, only oncornavirus reverse transcriptase is capable of extensively transcribing natural RNA templates in the absence of exogenous primer.

Significance to Biomedical Research and the Program of the Institute: These studies are providing an important insight into the mechanism by which RNA tumor viruses bring about malignant transformation, and perhaps will lead to significant advances in the understanding of the causation and control of human neoplastic disease. The contractor has made the important finding of no detectable difference in the amount of MTV-specific DNA between the GR (high incidence of MTV and breast cancer) and C57BL/6 (low incidence) strains of mice, as well as no detectable difference in the amount of avian tumor virus specific DNA in normal or transformed chicken cells. This suggests that all cells may contain tumor viral information (viral oncogene hypothesis) and that cancer is caused by the "switch-on" of the ubiquitously present oncogene.

Proposed Course: The polymerase of RSV will be purified to absolute homogeneity and its precise polypeptide constitution determined. The sequence of the RNA primer molecule associated with 70S RNA will be studied in sufficient detail to permit definitive identification. Various oncornavirus RNA's will be used as template for RSV polymerase and examined for preferential transcription of limited, homologous regions. The transcription of heterologous natural RNA's (e.g., poliovirus) will be studied with respect to extent of transcription, size and secondary structure of the transcripts and mechanism of initiation. The mechanism(s) by which isatin B-thiosemicarbazone inactivates both the reverse transcriptase and the infectivity of RSV will be examined in an effort to further implicate the enzyme in the biological activity of the virus. Transcription of natural RNA's (RSV, polio) by various cellular DNA polymerases will be studied with respect to primer requirement, extent of transcription and size and secondary structure of the transcript, all in an effort to accumulate further evidence that viral reverse transcriptase is unique among all known DNA polymerases. The assay of virus-specific DNA in normal and infected cells will be extended by means of a new technique to include sequences representative of the entire viral genome. Further evidence for covalent integration of viral DNA into host chromosome will be sought. The mechanism by which the expression of viral genetic information is controlled (repressed, partially or completely) in normal cells will be studied, principally by examining the relative extent to which viral DNA is transcribed in various normal cells, and by seeking factors which might influence that transcription.

Date Contract Initiated: June 2, 1971

Current Contract Level: $77,500

CALIFORNIA, UNIVERSITY OF (NIH-NCI-E-71-2173)

Title: Studies on the Structure and Replication of Viruses and Mechanisms of Regulation

Contractor's Project Directors: Dr. Howard K. Schachman
Dr. Peter Duesberg

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: (1) To study the RNA subunits and the replication of RNA tumor viruses. (2) To study mechanisms of cell regulation including transcriptional control by a satellite virus. (3) Electron microscopic studies of RNA viruses.

Major Findings: (1) The validity of the hypothesis that the a subunit of 60-70S RNA may be responsible for the transforming ability of avian tumor viruses was tested further. In collaboration with Dr. Peter Vogt (Contract No. NIH-NCI-E-72-2032), it was shown that several nontransforming avian tumor viruses such as RAV-2, subgroup B and RAV-7, subgroup C lack the a subunit. Using T5, a transforming mutant of RSV which is temperature-sensitive regarding the transformed state of the cell but not with regard to virus growth, it was found that the a subunit was present in T5, regardless of whether it was grown in transformed cells (at the permissive temperature) or in nontransformed cells (nonpermissive temperature). It was concluded that the a subunit is not the result of the transformed state of the cell but may directly or indirectly be involved in transformation, thus providing evidence for viral genes (oncogenes) being specifically responsible for the neoplastic state.

(2) The ratios of a and b subunits in stocks of transforming viruses varied on passage in tissue culture. In all cases the b subunit class increased at the expense of the a. Nontransforming viruses arose spontaneously from a transforming progenitor (Schmidt-Ruppin RSV) on passage in tissue culture. It thus appears that the RNA of RNA tumor viruses exists in subgenomic fragments like the RNA of influenza virus or Reo virus, and that varying RNA subunits may be selected from exchangeable replicating pools in the infected cell.

(3) It was found that the relatively high template activity of 60-70S RSV RNA is lost after heat-dissociation into 30-40S subunits. It was concluded that the partial double-strandedness of 60-70S RSV RNA, which holds the subunits together, is responsible for its high template activity. This model has been tested by attempts to restore the partial double-strandedness of heat-dissociated RSV RNA.

No success has been achieved in reversing the 60-70S → 30-40S conversion of RSV RNA, as yet, but it has been possible to restore the high template activity of RSV RNA after heat-dissociation by adding oligodeoxynucleotides (oligo dT, oligo dC, oligo dG, but not oligo dA).

(4) An RNase-resistant adenylate (A)rich sequence was found in the 60-70S RNA of Rous sarcoma virus and Rauscher murine leukemia virus. The A-rich sequence contains about 10% of the A of the viral RNA. The molecular weight of the A-rich sequence prepared by exhaustive digestion of 60-70S RSV RNA with pancreatic and T1 RNase was estimated by velocity sedimentation and gel electrophoresis to be about 60,000. After heat or formaldehyde dissociation of the 60-70S RSV RNA complex, A-rich sequences remain linked to the major 30-40S RNA subunits. It was concluded that each 30-40S subunit contains as part of the polynucleotide one or two A-rich sequences. (The finding of A-rich sequences has been confirmed by Dr. Maurice Green at St. Louis University [Contract No. PH43-NIH-NCI-E-67-692] and suggests possibilities for specific molecular and biochemical modification of the A-rich sequences. Another contractor, Dr. Tennant at AEC [Contract No. NIH-NCI-E-(FS-13)] found that synthetic poly A added to the media, specifically inhibited Moloney virus replication in cultured mouse cells.)

(5) A selection method for isolating phenotypically normal cells in cultures of transformed cells has been developed. Normal cells have greater adhesiveness to culture dishes. Treatment of mixed cultures with small doses of trypsin and EDTA, therefore, detaches transformed cells selectively leaving clones of revertant normal cells which can be cultured further.

(6) Dr. Calendar's group has been studying the life cycle of satellite phage P4, which depends on temperate coliphage P2 for help in multiplication. P4 can trans-activate helper genes from a repressed P2 prophage, while the normal cell repressor system remains intact. Their data suggest that translational control as well as transcriptional control may be involved in prophage trans-activation. The mechanism of this activation may be relevant to oncogenesis, if carcinogenic viruses and chemicals act by inducing repressed genes present in the host, which is one of the postulates of the viral oncogene hyposthesis.

(7) It has been found that the several pieces of RNA comprising the genome of influenza virus (WSN strain) and the RNA of avian sarcoma virus (B77 strain) both terminate at the 3'-end predominantly in uridine.

Significance to Biomedical Research and the Program of the Institute: Basic research on RNA tumor virus structure and replication, as well as studies on mechanisms of cell regulation, may provide the basis for understanding viral carcinogenesis which may ultimately lead to the control of human cancer. Studies on the a subunit of 60-70S RNA may lead to localization of the virally transmitted oncogenic information. Studies of the A-rich sequences in tumor virus RNA, may provide a handle for biochemical modification of the RNA.

Proposed Course: (1) Continuation of investigation of avian tumor virus strains to see if most or all transforming strains lack it. (2) Further chemical analyses of the a and the b subunits. It is planned to determine by analysis of the oligonucleotides obtained after RNase T1 digestion, whether and how much the a subunit is different from the other viral RNA subunits. (3) The dependence of the template activity of 60-70S RSV RNA on its subunit structure will be investigated by controlled dissociation of the 60-70S RNA and by analysis of the DNA made by the viral DNA polymerase in the presence of the dissociated template. (4) Nuclei have been isolated using the method of lyophilization of cells followed by homogenization and centrifugation in non-aqueous solvents. These nuclei have high DNA and RNA polymerase activities; they will be used in cell-free synthesis studies to look for virus-specific sequences. (5) Further attempts will be made to isolate satellite phage P4 mutants, in hopes of isolating a large collection of mutants defective in trans-activation of helper genes. Changes in the host RNA polymerase after P4 infection are being assayed. Purification of RNA polymerase by a sizing technique will be attempted and it will then be analyzed for electrophoretic changes and for phosphorylation. The activity of RNA polymerase on P2 templates will be determined. P2 mRNA species may be of a different size and structure when induced by satellite phage P4. This proposition will be tested by electrophoretic and hybridization analysis of mRNA produced in P4-infected P2-lysogenic cells. P4 may modify the host translational machinery; electrophoretic changes or phosphorylation in ribosomal proteins will be analyzed. (6) A problem in detailed electron microscopy is the damage to tumor virus structure occasioned by the manipulations necessary for isolation and purification of the virions. It is known that fibroblasts inoculated with Rous sarcoma virus will, under appropriate conditions, elaborate a great number of virions which remain in the near vicinity of the cells and exterior to their plasma membranes. A technique is being perfected wherein infected cells are grown on films suitable for electron microscopy. Elaborated virions will spread out from the cells and, it is hoped, they can be gently washed of adhering material, negatively stained,

and observed in the electron microscope. In this manner it may be possible to observe structural detail in the complete absence of conventional purification steps.

Date Contract Initiated: June 29, 1971

Current Contract Level: $150,000

CALIFORNIA, UNIVERSITY OF (NIH-NCI-E-70-2048)

Title: Comparative Leukemia and Sarcoma Viral Studies

Contractor's Project Director: Dr. Leo K. Bustad

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To further study the two simian Type C viruses (woolly monkey sarcoma and gibbon lymphosarcoma) which were first isolated in this laboratory.

Major Findings: (1) Woolly Monkey Fibrosarcoma (SSV): (a) SSV-infected bonnet monkey cells, cultivated in roller bottles, are yielding significant amounts of SSV virus. Several other cell lines of human, bovine, and simian origin also support SSV replication. (b) Bovine cells infected with woolly monkey sarcoma virus were inoculated into the autochthonous host at 10- to 14-day intervals as viable and freeze-thawed disrupted cells. Serum samples taken prior to inoculation and 6 weeks later were tested for antibody against the woolly monkey sarcoma virus by immunodiffusion. A positive precipitin band developed with a serum sample taken 6 weeks post-inoculation. (c) Radioimmune precipitation assays using SSV replicated in bovine thymus cultures were positive for bovine anti-SSV antisera but negative for bovine anti-FeSV antisera, indicating that SSV-infected cultures are free of FeSV antigens and that SSV and FeSV do not possess common envelope antigens. (d) Cellular and cell-free materials from tissue culture were inoculated subcutaneously into two newborn cotton-topped marmosets. Nodules (7 to 13 mm diameter) developed at the site of inoculation within 12 to 14 days. However, nodules regressed within 3 weeks. One nodule detected 28 days after inoculation reached 1 cm in diameter and has remained that size for 4 months. Blood samples from each animal have been examined monthly and no significant abnormalities have been noted. All inoculated monkeys are being held for continued observation.

(2) Gibbon Lymphosarcoma (SLV): (a) The cell line initiated from the gibbon tumor tissue remains the best producer of SLV, although bovine, African green monkey kidney (AGMK),

and human cell lines support low levels of SLV replication. (b) Transmission of the gibbon lymphosarcoma was tested in neonatal squirrel monkeys and pigs. Three of four newborn squirrel monkeys inoculated with gibbon lymphosarcoma tissue culture cells had a transient enlargement of the regional lymph node draining the site of inoculation. No signs of neoplasia have been observed during the 4 to 5 months following challenge; regular examination of blood samples has revealed no abnormalities. All monkeys remain under observation. Three of eight inoculated pigs were sacrificed 4 months after injection and found to have slightly enlarged mesenteric lymph nodes; histopathologic diagnosis was lymphoid hyperplasia. No other lesions were detected. Surviving pigs have shown no signs of neoplasia in the 7 to 9 months since inoculation and remain under observation.

Significance to Biomedical Research and the Program of the Institute: The finding at this laboratory of two Type C viruses associated with tumors of different primate species is evidence that the higher animals, including man, are likely to be among the growing number of species harboring oncornaviruses. Since monkeys and man are closely related phylogenetically, the proposed studies, which are oriented toward characterization of the primate viruses and seeking possible relation with human tumors, are of direct relevance in establishing the etiology of human cancer.

Proposed Course: (1) Characterize in greater detail the molecular components of the woolly monkey fibrosarcoma and gibbon lymphosarcoma viruses by analysis of enzymes, structural proteins and nucleic acid. (2) Determine the *in vitro* and *in vivo* biological activities of woolly monkey and gibbon lymphosarcoma viruses. (3) Initiate serologic studies for the detection of antigenic components in spontaneous tumors of simian and human origin that may be common to know simian RNA Type-C viruses. (4) Continue efforts to isolate oncogenic agents from spontaneous tumors of primates and complete limited studies on canine systems. (5) Complete studies on humoral antibody response in cats to the leukemia-sarcoma virus complex.

Date Contract Initiated: November 16, 1969

Current Contract Level: $550,000

EINSTEIN MEDICAL COLLEGE (PH43-NIH-NCI-E-65-612)

Title: Genetic and Immunological Factors in Viral Leukemogenesis

Contractor's Project Director: Dr. Frank Lilly

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To gain a better understanding of the known or suspected genetic factors that play roles in the cancerous process.

Major Findings: (1) Studies of two inbred backcross systems show that within linkage group II of the mouse, the gene order is: theta - tk - d - Mod-1 - Fv-2, where these five loci code for, respectively, an antigen on thymic lymphocytes, tail kink anomaly, dilute coat color, an enzyme in the malic acid metabolism series, and susceptibility to Friend (and Rauscher) viruses. (2) AKR mice show a high incidence of spontaneous leukemia at around 8-11 months of age, whereas DBA/2 and (AKR x DBA/2) F_1 mice show no leukemia at this age; however, when these mice received methylcholanthrene paintings at 3 months, then (a) the leukemia in AKR mice occurred significantly earlier and (b) both DBA/2 and F_1 mice showed a marked incidence of leukemia, but this occurred at a rate still slower than unpainted AKRs. The results indicate that spontaneous leukemia in the AKR strain is recessive in this cross. However, the DBA/2 susceptibility to carcinogen-induced leukemia (and their resistance to carcinogen-induced skin tumors) is dominant in this same cross. (3) BALB/c and DBA/2 mice were previously not known to differ in their response to infection with F-B mouse leukemia virus; it is now found that 1-2 days after injection, copious virus bands (in sucrose gradients) appear in the sera of BALB/c mice, while there was little such virus in the sera of identically treated DBA/2 mice. C3H mice resemble BALB/c's in this respect, and D2.R^S mice resemble DBA/2 mice, which shows that the Fv-1 locus is not the determinant of this phenomenon. (4) This laboratory had previously reported that transplantation antigen H-2.31 disappears almost completely from spleen cells of BALB/c mice in the late stages of the Friend virus disease. It is now noted that there appears to be a gene present in some strains of mice which represses the expression of this antigen on erythrocytes (but which has no effect on the same antigen on lymphocytes) of mice known to have the H-2.31 antigenic determinant. (5) In studies on the genetic factors involved in the immune response to the H-2.2 histocompatibility antigen, it appears that two genes are involved in this

trait: one linked to H-2 itself (this gene is present in A mice) and the other at a locus independent of H-2 (this gene is present in BALB/c mice).

Significance to Biomedical Research and the Program of the Institute: One of the basic facts about tumor biology is that genetic mechanisms of the host exert strong control over the expression of oncogenicity. Dr. Lilly's work represents one of the few indepth studies being done on the genetics of susceptibility to viral oncogenesis. The knowledge derived from this study and the mouse strains developed will have broad applications both to the SVCP Program and the general effort in virus-cancer problems.

Proposed Course: Studies of: (1) The mechanism of the H-2 (histocompatibility genes) effects on leukemogenesis. (2) The mechanism of the Fv-1 and Fv-2 (susceptibility to Friend virus genes) effects on leukemogenesis (including determining if Rgv-2 is the same as Fv-1). (3) Map Fv-1, is Fv-2 related to H-7 (histocompatibility gene)? (4) Abelson lymphosarcoma virus (looking for the strain distribution of response, and if the virus consists of a helper plus a defective pathogenic component). (5) Friend virus radio-labeled (in vivo), to examine the component parts of the virus and its alternate forms of helper virus (N-, B-, or NB-tropic). (6) Immune response gene(s) for H-2.2.

Date Contract Initiated: May 13, 1965

Current Contract Level: $180,000

FLOW LABORATORIES, INC. (NIH-NCI-E-71-2097)

Title: Studies of Type C Viruses and Herpesviruses in Relation to Oncogenic Potential

Contractor's Project Director: Dr. Raymond V. Gilden

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To conduct immunological and serological studies of oncogenic Type C RNA viruses and the herpes-like viruses associated with neoplasia. Virus proteins and RNA are purified in quantity, their structure analyzed, and the major protein used to prepare mono-specific antisera for immunological studies.

Major Findings: (1) Type C Viruses: (a) The major internal virion antigen (gs-1) of the RD 114 virus (candidate human Type C virus) has been purified by isoelectric focusing. The isoelectric point is 9.1, thus clearly distinguishable from the homologous protein of other mammalian Type C viruses; e.g., cat, 8.3; mouse, 6.7; hamster, 6.9; rat, 8.6. The purified protein was used to immunize guinea pigs and the serum showed a high degree of specificity for RD 114 in both complement-fixation and gel diffusion assays. Highly sensitive radioimmunoassay inhibition tests further indicated the lack of cross-reaction between RD 114 and FeLV, and also indicated qualitative differences between the gs-3 determinant of RD 114 and the non-primate Type C viruses. Gel diffusion tests with a polyvalent goat anti-FeLV serum (courtesy of Dr. Roger Wilsnack) also revealed two other FeLV specific proteins in FeLV which were not found in RD 114. (b) Successful rescue of the MSV genome from non-producer hamster tumor cells was accomplished by a cell fusion technique using Sendai virus. The rescued virus produced clear foci of transformed cells selectively on one of six human cell strains tested. (c) The gibbon and woolly monkey Type C viruses were found to possess the gs-3 determinants in gel diffusion tests (in collaboration with Dr. T. Kawakami); thus, this continues to be a distinctive feature of all mammalian viruses of this family. These viruses did not react with the species-specific antisera for cat, hamster, mouse, and rat viruses. The gibbon virus was also used to rescue the MSV genome from non-producer hamster tumor cells. (d) Attempts to demonstrate antibodies in 20 normal and tumored cats to the major internal protein of FeLV using radioimmunoassay procedures were unequivocally negative. This procedure gives at least 100-fold higher titers with control positive serum from immunized guinea pigs than complement-fixation tests. This test supports the earlier CF results indicating that animals are immunologically tolerant to their species-specific gs antigen. (e) Uridine is the 3' hydroxl terminal of the 70S RNA of all known Type C viruses. An estimate of the molecular weight of viral RNA based on terminal tritiation is ~2.5 x 10^6, consistent with a four subunit structure. (f) Antibody to the reverse transcriptase was obtained from rats bearing an AKR virus induced lymphosarcoma. This serum specifically inhibited mammalian Type C enzyme but not snake, chicken, or normal cell polymerases. A serum with a high degree of specificity for inhibition of the DNA dependent polymerase activity was obtained by immunization with purified feline virus enzyme. This serum exhibited the same species specificity as the tumored rat serum. (g) This contract has demonstrated and produced purified gs antigens from 10 different animal tumor viruses. These have been made available and used for extensive natural history studies of Type C RNA tumor viruses in mice, hamsters, rats, and

cats. Additional similar reagents are being produced for the candidate human RD 114 virus, the woolly monkey, and the gibbon ape virus.

(2) Herpesviruses: (a) Definitive evidence was provided for the persistence of EB virus in repressed state in virus "negative" lymphoblastoid cells. Virus was induced from the negative Raji cell by first making the cells resistant to BrdU and then removing the drug. Following drug removal, virus specific antigens and eventually complete virion appear in ~1% of cells. The carrier line P3HR-1, which produces viral antigens but not particles when grown in BrdU. After removal of BrdU, a low percentage of cells are able to incorporate thymidine and combined immunofluorescent and audioradiographic studies showed that it is these cells that synthesize viral antigens. The drug resistant cell lines offer a unique opportunity to study the mechanism of virus derepression since only thymidine kinase (TK) positive cells produce virus; thus, the high background of normal cell DNA synthesis (99% of the cells) is effectively screened out using thymidine or its analogues to study the nature of the DNA made after drug removal. (b) The immunoferritin technique has been used for differentiating antigens associated with Herpes simplex virions and for following the course of virus antigen production in situ. Using the immunoferritin technique, the specificity of heterophile positive infectious mononucleosis sera for EB virus was localized in the γM fraction, in contrast to "normal" human sera where anti-EB virus activity is localized in the γG fraction. This supports an etiologic role for EB virus or a serologically related virus in infectious mononucleosis.

Significance to Biomedical Research and the Program of the Institute: This project is deeply involved with the very important problem of determining whether viruses recovered from tumors are oncogenic in man. Since man will not be used as an experimental recipient, it is necessary to gain proof of oncogenicity by other means including seroepidemiological surveys for virus, virus antigen, and/or specific antibody. The contractor is producing and studying the virion antigens of Type C viruses and herpesviruses associated with neoplasia. Mono-specific antisera are being produced to these antigens, which are then used for seroepidemiologic studies carried out in a number of different laboratories; there is very active collaboration with the in-house VCB program, and with many other SVCP contractors (e.g., USC [PH43-NIH-NCI-E-68-1030], Microbiological Associates [NIH-NCI-E-70-2068], Univ. of Calif., Davis [NIH-NCI-E-70-2048], Princeton [NIH-NCI-E-71-2372], and Pfizer [NIH-NCI-E-70-2080]).

Proposed Course: (1) Large scale production of RD 114 virus and one other primate virus and preparation from them of

purified gs antigen, envelope antigens, and polymerase under rigid quality control. (2) Continued characterization of RD 114 virus including gs and envelope proteins. (3) Production of specific RD 114 gs antiserum and beginning of surveys of human materials for RD 114 gs antigen. (4) Continued production and characterization of other gs proteins by amino acid sequence analysis. (5) Purification of RD 114 virion RNA dependent DNA polymerase and production of specific antisera. (6) Search for specific non-virion proteins in infected and transformed cells. (7) Structure and terminal nucleotide sequence of viral RNA, and transformed cell virus-specific RNA. (8) Analysis of the sugar transport marker of sarcoma virus transformed cells. (9) Study of mechanism of EB virus derepression in lymphoblastoid cells. (10) Similar studies with fibroblasts in which Type C's can be evoked. (11) Investigations of herpes and Type C viruses using immunoferritin, immunofluorescence and enzyme labeled techniques, involving a subcontract with Dr. Konrad Hsu at Columbia University.

Date Contract Initiated: February 1, 1971

Current Contract Level: $2,410,000

THE JACKSON LABORATORY (PH43-NIH-NCI-E-67-744)

Title: Natural Occurrence of RNA Tumor Viruses (Genomes) and Host-Gene Control of Their Expressions

Contractor's Project Director: Dr. Hans Meier

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: The primary objective of this contract is to achieve an understanding of the mechanisms underlying the genetic determination of susceptibility and resistance to cancer and the RNA tumor viruses. There is little doubt that the expression of viral functions is in large part controlled by host genes. The Jackson Laboratory is a unique source of highly inbred mouse strains. These are used to define specific gene influences on Type C RNA virus/genome/tumor expressions under natural conditions, and the influence of environmental and other factors (carcinogens, aging) on host gene controls of oncogene and virus expressions.

Major Findings: (1) Host genetic rescue of murine leukemia virus: Complete infectious murine leukemia virus has been recovered by host genetic rescue in hybrids of two virus-free

lines of mice. Two autosomal dominant genes are required for the presence of complete virus. (2) Host-gene control of Type C RNA tumor virus: Genetic studies were aimed at elucidating the mechanism of inheritance of the group-specific antigen of the murine leukemia virus. Two approaches have been used. First, a classical Mendelian hybridization experiment was performed, utilizing mice of the high-leukemia AKR strain and the low-leukemia C57L strain, the first filial (F_1) generation hybrids, the second filial (F_2) generation hybrids, and the backcross generations of this cross. The second approach used a number of partially inbred lines derived from the F_2 generation of the same cross. The results of these studies demonstrated specific genetic regulation of expression of group-specific antigen. Genes ($Mlv\text{-}1^a$ and $Mlv\text{-}2^a$) permissive to the expression of the antigen are dominant or semi-dominant to their nonpermissive alleles ($Mlv\text{-}1^b$ and $Mlv\text{-}2^b$). (3) Chemical co-carcinogenesis: Experiments clearly indicate the host-gene control of both tumorigenesis and expression of function of endogenous Type C RNA tumor viruses. They lend support to the concept that cancer results from endogenous (host-genetic) or exogenous (acquired, carcinogen-induced) stimulation of an inherited viral genome. (4) Recombinant inbred (RI) lines, a new approach in genetic analysis: Two unrelated inbred strains are crossed and a number of inbred lines are developed from the F_2 generation by close inbreeding. The rationale for this procedure is that linked genes will tend to become fixed in the same combinations (parental) as they entered the cross. Unlinked genes are randomized in the F_2 generation and are therefore equally likely to be fixed in parental or recombinant phases. RI lines are particularly useful for analysis of (a) segregation, (b) linkage, (c) gene functions, and (d) mutations. Other advantages are (e) tissue from different individuals within an RI line may be pooled, without introducing genetic heterogenicity, (f) genetic differences tend to be maximized due to the elimination of heterozygotes, (g) the recessive characteristics of both parental strains are expressed in RI lines, (h) once RI lines are established, the time delay required for production of the segregating generations can be avoided, (i) genetic associations, due to either pleiotropy or linkage, may be detected between traits expressed in utero and adult characteristics, male and female limited traits, and traits that require killing individuals at different stages or require different pretreatments, and (j) the F_1 hybrid of the parental lines will accept tissue or organ grafts from any of the RI lines. (5) Genetic relationship between aryl hydrocarbon hydroxylase inducibility and chemical carcinogenesis: Inbred strains of mice show differential inflammatory reactivity and susceptibility to tumor induction following the topical application of polycyclic hydrocarbons. Strains also differ in the extent to which aryl hydrocarbon hydroxylase activity

is induced by these compounds. Differential inflammatory response and hydroxylase inducibility are genetically controlled by alleles at the In and Ahh loci, respectively. Genetic analyses and strain surveys have established that the In and Ahh loci are identical and that skin ulceration in response to topical carcinogen requires that aryl hydrocarbon hydroxylase activity be inducible.

Significance to Biomedical Research and the Program of the Institute: This program has contributed much of the basic data concerning the genetic determinants of oncogenesis and the natural expressions of the endogenous Type C RNA virus. It has pointed up the overwhelming influence of genetic predisposition in the development of natural cancer and susceptibility to environmental carcinogens. Contractor has developed sophisticated systems for defining and locating the genes and loci involved in murine oncogenesis, and virus and antigen expression, and has rescued complete virus through gene complementation by hybridization of two virus-free mouse strains. Information derived from this research bears direct relevance to the human cancer problem; definition of the heritable nature of cancer is essential to eventual control or prevention of the disease. These well defined murine systems and tissue cultures derived from them provide valuable vehicles for assay of environmental carcinogens and experimental therapeutic measures.

Proposed Course: (1) Development, characterization, and uses of recombinant inbred lines. (2) Study of genetic control of endogenous murine leukemia virus. (3) Study of association of the viral group-specific antigen with tumor development. (4) Genetic analysis of a dominant gene inhibiting expression of the group-specific antigen. (5) Mapping of viral structural genes by use of defective sarcoma virus mutants: complementation classes. (6) Additional marker studies for mapping structural genes of viral components. (7) Induction of complete or partial virus synthesis by drugs. (8) Chemical co-carcinogenesis studies. (9) Marker studies (immunogenetic and biochemical). (10) Study of genetic control of embryonic and post-natal, normal and abnormal cell proliferation. (11) Study of the effect(s) of hormonal stimulation of RNA metabolism in dwarf mice, and (12) host genetic rescue of murine leukemia virus.

Date Contract Initiated: May 2, 1967

Current Contract Level: $382,000

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-NIH-NCI-E-67-697)

Title: Research on Animal Tumor Viruses

Contractor's Project Director: Dr. Robert M. Nims

Project Officer (NCI): Dr. Gary Kelloff

Objectives: The contract involves a long-term, comprehensive study to determine the natural incidence of Type C RNA virus expression and of cancer in nine strains of inbred mice and one strain of rat. The effort includes life time surveillance of defined strains for spontaneous neoplasms; serological testing of normal and tumor materials for the presence of Type C viruses and antigens; characterization of viral isolates as leukemia and cancer inducing agents; and the administration of hormones and carcinogens to study their effects on tumor and virus expression.

In addition to the research described above, the contract constitutes an important service to the SVCP. For specimens submitted for testing by NCI supported scientists, the contractor provides services which include storage and inventory of biological specimens, bioassays of leukemia and related viruses in a number of species, tissue culture and serological assays; also an extensive histopathology laboratory and pathological diagnostic service.

Major Findings: (1) Type C viruses isolated from spontaneous neoplasms of the BALB/c colony under study have been characterized in the CoMuL, SPAT, and XC tests as mostly B-tropic; viruses isolated from normal mice are mostly N-tropic, or a mixture of N- and B-tropic. (2) Studies have delineated the natural history of the Type C genome. Experiments in these aging colonies are now being focused on the tumor-inducing effects in vivo of the wild mice type viruses recovered from spontaneous tumors as well as from tumors induced by radiation, chemicals, and IdU. Preliminary results show that when injected into newborns the wild type viruses produce leukemia within 12 to 15 months. (3) Long-term cell-free passages of spleen and tumor tissues from spontaneous neoplasms have a highly significant association between virus isolation (SPAT and CoMuL tests) and induction of reticuloendothelial system neoplasms. (4) Streptonigrin (SN) has proven a potent inhibitor of Type C RNA virus replication with 87% inhibition achieved after 48 hours treatment and 98% after 120 hours. (5) A focus-forming sarcoma virus was recently isolated from a spontaneous sarcoma. This isolate produced diffuse transformation on BALB/c embryo cells, low frequency transformation on NIH Swiss embryo cells, and localized MSV-like foci on Fischer rat cells; the supernatant from transformed

embryo cells produced tumors in newborn BALB/c mice within 3 weeks after inoculation. This virus is of particular interest since it represents only the second sarcoma virus isolated from the mouse (the first was the FBJ virus, derived from a murine osteosarcoma). (6) Transplantation of two Fischer rat spontaneous leukemias to ACl rat newborns was successful. (7) Studies with Dr. Frank Portugal (NCI) showed that certain MuLV infected tissue culture cell lines have a natural repressor for MuLV replication in vitro and sarcomagenesis by MSV in vivo. The repressor is not an interferon or interferon inducer. (8) Experiments to determine the effect of extradiol and ovariectomy on uterine gs antigen expression have not revealed any appreciable differences between normal and hormone-deprived groups and are being terminated. A second series of hormone studies designed to elucidate the effect of diethylstilbesterol administered to pregnant Snell mice on cancer in their offspring (modeled after recent reports in humans) is nearing completion. (9) One of the major service efforts provides histopathological backup to this and other contract research. As an example of the scope, 3,600 slides were processed in October, 1971; two-thirds of the specimens originated from this contract program, and the remainder from other SVCP contracts.

Significance to Biomedical Research and the Program of the Institute: The service aspects of this contract are in direct support of the SVCP. Services include reagent production, serological tests, and histopathology for several laboratories. The surveillance of a number of animal colonies for their lifespan provided many of the key insights into the natural history of the oncogene. These virus-defined colonies are now being studied to determine how various endogenous and exogenous factors influence the carcinogenic effects of chemicals, radiation, etc. They also provide an excellent resource for isolation of natural inhibitors, and studies of interferon and vaccine effects on natural tumors.

This contract contributed importantly in ruling out the likelihood of vertical spread of Type C viruses in mice and also has provided much of the large body of information implicating the Type C genome in normal embryogenesis.

Proposed Course: (1) Assessment of oncogenic potential and in vivo growth capabilities of plaque purified B- and N-tropic MLV viruses isolated from normal and neoplastic cells. (2) Analysis of long-term neoplasia induction study involving the effect of age on tumor and virus induction by radiation or chemicals. (3) Attempt to produce high titered human interferon, study of interferon levels in germ-free and conventional mice, and study of interferon prophylaxis of spontaneous and chemically induced neoplasms. (4) Application

of specific immunofluorescent reagents to the study of the natural distribution of Type C RNA viruses in normal and neoplastic tissues. (5) Production of purified MLV envelope and gs antigens as immunogens for natural history and experimental studies. (6) With Dr. Frank Portugal (NCI), further characterization of effect of natural repressors and antibiotics on murine leukemia virus replication. (7) Determination of tumorigenicity of chemically transformed rat cell preparations in Fischer rats. (8) Continuation of providing services for other SVCP contracts.

Date Contract Initiated: November 15, 1961

Current Contract Level: $719,500

MICROBIOLOGICAL ASSOCIATES, INC. (NIH-NCI-E-70-2068)

Title: The Roles of Viruses and Chemicals in the Etiology of Cancer

Contractor's Project Director: Dr. Riley Housewright

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: Project A - Dr. Johng S. Rhim and Project B - Dr. Aaron E. Freeman: These projects are concerned with the development, evaluation, standardization and application of sensitive in vitro assay systems for studies of carcinogenic agents found in the environment to define the mechanisms of chemical and viral carcinogenesis in vitro, and to correlate these findings with in vivo studies of Projects C and D. Test systems include rat, mouse, hamster, and human tissue cultures at advanced levels of subculture, both uninfected and infected with various Type C RNA viruses. Materials under study include known and suspected carcinogens, derived from the natural environment (smog and tobacco smoke residues, food additives, hazardous industrial products) and provided by various collaborative contracting groups, particularly the program at USC (Contract PH43-NIH-NCI-E-68-1030), and large numbers of coded carcinogens and their analogs provided by Dr. John Weisburger (NCI).

Project C - Dr. Padman S. Sarma: This project is concerned with the development of sensitive in vitro assay systems for studying the prevalence of the naturally occurring feline, rat, avian, and other animal tumor viruses, utilizing the methodology previously developed under this contract. Dr. Sarma has been a primary contributor in designing test systems for rescue and detection of defective and endogenous tumor virus genomes utilizing trans-species rescue cell co-cultivation and sophisticated serological

techniques. These approaches are being intensively applied to various animal tumor systems having covert (switched-off) viral genomes in efforts to "turn on" overt expressions of the genomes, the ultimate objective being to apply successful methods to studies of human cancer.

Project D - Dr. Carrie E. Whitmire: This project tests RNA virus genome derepression by carcinogens in vivo, leading to gs antigen, infectious virus and/or tumor induction. The animal systems used include a variety of high and low leukemic incidence strains of mice, hamsters, and rats, including those strains from which tissue cultures were derived for Projects A and B. In addition to a variety of known laboratory carcinogens, suspected environmental carcinogens such as smog and tobacco products are included in test protocols concomitant with in vitro studies under Project B. Evaluation of carcinogenic effects is compared using different doses, routes of inoculation, age of animals, maternal parity influence, and other physiological and environmental factors which appear to be associated with susceptibility to tumor induction.

Project E - Dr. M. Lee Vernon: This project provides electron microscopy support to Projects A-D. The chief objective is to determine the prevalence of Type C RNA tumor virus particles in tumor tissues as well as normal embryonic and postnatal tissues taken at various ages.

Project F - Dr. John C. Parker: This project provides serological support to this and other VCB contracts.

Major Findings: Project A: Transformation was induced by extracts of city smog and tobacco smoke fractions in mouse embryo cells chronically infected with AKR leukemia virus, but uninfected mouse embryo cells were not transformed. The transformed cell lines produced tumors when transplanted into newborn homologous hosts.

A guinea pig embryo (GPE) cell line was found to be sensitive to chemical transformation. Also, GPE cells were transformed in vitro by Ki-MSV; the transformed cells contained both infectious virus and gs antigen.

Transformation of rat cells was achieved by the use of irradiated mouse cytomegalovirus (MCMV).

AKR virus infected NIH Swiss mouse embryo (ME) cells were readily transformed by chemical carcinogens (3MC, DMBA, and BP); this cell test system, when compared with rat and hamster systems proved to be the most sensitive test for carcinogenic chemicals.

Transformation by polyoma virus was facilitated by the use of established rat cell lines instead of primary or secondary cultures; transformation was further accelerated in rat cells chronically infected with RLV. The results suggest that the Type C RNA virus genomes provided not only oncogenic determinants for chemical in vitro transformation, but also for DNA virus cell transformation.

Polyoma transformed hamster embryo cells regularly led to hamster leukemia virus (HaLV) gs antigen after transplantation into newborn virus-free hamsters. Similarly, polyoma transformed NIH Swiss cells were shown to have Type C virus gs antigen when transformed cells were transplanted into virus-free NIH mice.

Project B: The synergistic activity of chemical carcinogenesis and Type C virus was studied in Fischer rat embryo cultures. Transformation appeared to be dependent upon a minimum level virus titer established in the rat cells at the time of chemical treatment. Cells treated with either agent alone or with chemical first, followed by viral infection after removal of chemical, remained normal.

The hamster Type C virus genome was shown to be ubiquitous in hamsters. In three hamster strains, tumors produced in vivo directly by inoculation of chemical carcinogens or by cells transformed in vitro by chemicals, polyoma virus or spontaneously, and tumor derived cell lines were frequently positive for Type C RNA virus by CF test.

Using a technique that separates cells according to their rate of attachment to glass, the reverse transcriptase activity of the candidate human Type C virus, RD 114, has been enhanced 30-fold. This virus has been adapted to grow in suspension cultures of a serial line of human prostate with a resultant four-fold increase in mature and immature Type C particles. Similar techniques have been successfully applied to the growth of woolly monkey and gibbon ape viruses in tissue cultures.

A series of chemical carcinogens and related inactive analogues have been tested for their ability to transform cells in vitro. In general, in vitro transforming activity was correlated with tumor production in vivo. However, a few "noncarcinogenic" agents did reveal transforming activity.

It has been shown that Type C oncogenic viruses were involved in chemically induced transformation in three separate systems: (a) Rat cells were not transformed by chemicals unless exogenous non-transforming MuLV was added. (b) Hamster cells were transformed by chemicals, but added HaLV decreased

the minimal transforming dose ten-fold. (c) Hamster cells transformed by chemicals produced tumors from which HaLV was regularly isolated.

Project C: RD 114 virus has the attributes of an infectious mammalian Type C virus and is unrelated to any of the various known avian and mammalian Type C RNA tumor viruses.

Endogenous latent Type C viruses of rats were activated in rat cell lines by treatment of cultures with 5-iododeoxyuridine and 5-bromodeoxyuridine.

The viral envelope antigens of feline Type C viruses responsible for viral infectivity were found to be responsible for induction of viral interference in the inoculated cultures against antigenically related challenge viruses. Three envelope antigens were detected which enabled classification of the feline Type C viruses into subgroups A, B, and C. The presently known members of the B and C subgroups, including three strains of feline sarcoma viruses, were found to be antigenic mixtures containing an A subgroup virus as one of the components. Virus purification of B and C subgroups was done by cloning techniques.

In rat cell lines releasing "defective" Type C particles, the viruses could not be rescued with defective murine sarcoma virus genomes. It appears likely that the cell lines, including three lines transformed by Rous sarcoma virus, contain the rat Type C virus in a "defective" state.

Prolonged passage of feline leukemia virus in human tumor cells (human rhabdomyosarcoma cells) did not alter the host range or envelope antigenic characteristics of the feline leukemia virus, suggestive of genetic recombination with a covert human Type C viral genome.

Project D: Studies of carcinogen induced tumors in various strains of inbred and random bred mice have provided a basis for strain selection and carcinogen dose to be used in future programs. While tumor incidence and latency is carcinogen dose dependent, there is considerable difference in the relative susceptibility of various strains. The incidence of gs antigen in the induced tumors is independent of the carcinogen or carcinogen dose used for tumor induction and reflects the natural gs antigen expression of the mouse strain; the tumors produced by carcinogens were "switched-on" in a high proportion of instances, while adjacent normal tissues remained "switched-off."

The phenomenon of mutual exclusion of leukemia and sarcoma neoplastic expression has been extensively studied in the AKR mouse strain. With sufficient doses of 3-methylcholanthrene

(3ME), subcutaneous tumors were produced which apparently prevented the expected spontaneous leukemias. Once the natural process of spontaneous leukemia induction had occurred, the resistance to subcutaneous sarcoma induction increased appreciably.

Studies with BALB/c and NIH Swiss mice of the effects of exogenous wild Type C RNA viral infection on 3MC induced sarcomas have shown the response to vary with the viral strain, the carcinogen dose, the mouse strain, the sequence of inoculation and the sex of the host.

Project E: In the last year almost 700 specimens were examined in the electron microscope for viral particles. A significant proportion of time has been devoted to studies of the RD 114 virus. Type C particles have now been observed in one or more hematopoietic tissues from embryos of 11 strains of mice while all strains were negative for Type C particles in muscle tissue at all gestational periods examined. Type B particles have been found in abundance in mammary tissues of wild mice 6 and 17 days in term, as well as in mammary tumors of the Claude substrain of BALB/c mice.

Project F: During the last year over 10,000 specimens were received on which over 20,000 serodiagnostic tests were performed. Many of the specimens were wild mouse sera received from USC (Contract PH43-NIH-NCI-E-68-1030) which were assayed for lymphocytic choriomeningitis and polyoma antibody, as well as for other naturally occurring mouse viruses.

Significance to Biomedical Research and the Program of the Institute: This contract represents a key part of SVCP's total effort. It has helped establish the role of the Type C viral genome in many feral and laboratory animal and cell systems. A number of test and assay systems developed under the contract are now used by laboratories throughout the world for investigating and characterizing RNA tumor viruses and their oncogenic and antigenic expressions.

Dr. Sarma's group has contributed no fewer than five highly sensitive serological (complement fixation, COFAL, COCAL, Neutralization, and interference) tests for field and laboratory use. Dr. Freeman's and Dr. Rhim's in vitro, rat, mouse, and hamster cell culture systems have provided new systems for sensitive and rapid assays of carcinogenic compounds in the natural environment. It is now possible to rapidly screen suspect compounds such as can be fractionated from smog and tobacco residues. The consistent correlations recently observed between in vitro transformation induced by the co-carcinogenic activity of RNA tumor viruses

and chemical carcinogens and in vivo tumor production by carcinogens have pointed up the feasibility of replacing current cumbersome, expensive and long-term in vivo bioassays with more efficient and sensitive in vitro systems. Dr. Whitmire's studies of the chemically induced expressions of the endogenous Type C viral genome are providing basic information on the effect of carcinogens in strains of mice having widely varied genetic susceptibilities to cancer. Dr. Vernon's EM studies have established a positive correlation of gs antigen content with the presence of Type C particles in various embryo organs. This correlation supports the concept of vertical transmission of the RNA genome. All these studies have furnished important evidence in support of the viral oncogene hypothesis.

There is extensive collaboration with numerous other groups supported by the SVCP; e.g., Microbiological Associates (PH43-NIH-NCI-E-67-697), USC (PH43-NIH-NCI-E-68-1030), California State Department of Public Health (PH43-NIH-NCI-E-68-997), and University of California (Naval Biomedical Research Laboratories [PH43-NIH-NCI-E-63-13]).

In summary, this contract is a core part of a coordinated comprehensive targeted research program to study the role of the Type C RNA virus genomes as determinants of ontogenesis and oncogenesis.

Proposed Course: Project A: (1) Currently available in vitro assay systems (particularly mouse cell) will be further used to screen the oncogenic potential of many additional environmental carcinogens. In addition, further efforts will be made to develop an in vitro assay system, utilizing human cell cultures. (2) KI-MSV transformed guinea pig embryo cells will be characterized. (3) Further studies on transformation of rat cells by exposure to irradiated cytomegalovirus are planned.

Project B: (1) Animal cell transformation systems developed in this laboratory will be used to identify and assay chemical carcinogens. (2) Efforts will continue using primate viruses to develop a human cell transformation system for identification and assay of chemical carcinogens. (3) Determination of the relationship between cell type transformed and histological type of tumor obtained by an in vitro transformation system utilizing "normal" epithelial cells. (4) Studies of transformation of virus infected cells cultured for many subcultures and comparison with spontaneous transformation in normal uninfected cells. (5) Utilization of labeled carcinogens to study the quantitative uptake, persistence and distribution of chemicals in cell culture.

Project C: Sensitive in vitro immunological and biochemical techniques are being developed for the quantitation of human and other primate candidate Type C viruses and antibodies to them.

Project D: In vivo studies will be begun on the effect of various viral inhibitors (interferon and active immunization with highly purified inactivated viral vaccines) on chemical carcinogenesis.

Other studies will include the relationship of genetic influences and natural viral genome expression on carcinogenesis in mice, rats, and hamsters. Studies will be undertaken to induce sarcomas in syngeneic mouse strains with viruses isolated from chemically induced sarcomas. The isolation of viruses from gs negative chemically induced tumors will be attempted using 5-iododeoxyuridine and 5-bromodeoxyuridine.

Project E: Electron microscopy will continue to search for the presence of Type C virus particles and other viruses and virus-like structure in normal and cancerous tissue and cultured cells.

Project F: Serodiagnostic laboratory support will continue to be provided.

Date Contract Initiated: February 1, 1970

Current Contract Level: $2,080,000

PRINCETON UNIVERSITY (NIH-NCI-E-71-2372)

Title: Studies on Surface Alterations in RNA Tumor Virus Transformed Cells

Contractor's Project Director: Dr. Max M. Burger

Project Officer (NCI): Dr. Gary Kelloff

Objectives: To study the surface alterations in transformed cells, as manifested by agglutinability with plant lectins.

Major Findings: Several RNA virus transformed tissue culture cell lines were investigated for increased agglutinability with plant lectins as was previously found by this contractor after transformation with DNA viruses. It was found that Rous virus (SR) transformed secondary chick embryo fibroblasts agglutinated slightly better than untransformed cells with jack bean agglutinin and that the increase was more pronounced with wheat germ agglutinin.

After treatment with purified hyaluronidase, cells agglutinated ten-fold better after transformation as compared with prior to transformation. That is essentially the same difference observed after transformation with DNA viruses.

When cells transformed with a temperature-sensitive Rous virus were shifted from the permissive to the nonpermissive temperature, they decreased their agglutinability and regained it only after shifting back to the permissive temperature. Decrease and increase in agglutinability were more pronounced if the cells were treated with hyaluronidase.

Some preliminary experiments have been carried out on Rauscher virus and Moloney sarcoma virus transformed mouse fibroblasts with Drs. C. Long and R. Gilden of Flow Laboratories (Contract No. NIH-NCI-E-71-2097). Increased agglutinabilities were found for both the producer and the non-producer lines as compared with the untransformed mouse parent cell lines. In this case, hyaluronidase seemed not to be necessary to observe sizeable differences in agglutinability.

Significance to Biomedical Research and the Program of the Institute: These studies aid in understanding the viral induced changes that occur in the molecular architecture of the cell surface upon transformation by RNA viruses. The surface changes of transformed cells are undoubtedly involved in the phenomena of unrestrained growth and metastasis of tumors.

Proposed Course: Continuation of work on mouse virus transformed mouse fibroblasts (together with Drs. C. Long and R. Gilden of Flow Laboratories), including examination of the effect of other agglutinins (in addition to wheat germ agglutinin) on these lines; the effects of trypsin and hyaluronidase and transformation by other RNA viruses will also be examined.

Date Contract Initiated: June 28, 1971

Current Contract Level: $56,898

SAINT LOUIS UNIVERSITY (PH43-NIH-NCI-E-67-692)

Title: Search for Viral-Specific Genetic Material in Human Cancers and Studies on the Mechanism of Oncogenesis by RNA and DNA Tumor Viruses

Contractor's Project Director: Dr. Maurice Green

Project Officer (NCI): Dr. Bernard Talbot

Objectives: This research program is aimed at understanding in detail the mechanism of cell transformation by RNA and DNA tumor viruses, applying new information on viral carcinogenesis and on the molecular biology of human cells directly to the problems of human cancer, and searching for inhibitors of polymerase that may control the expression of cancer specific genetic information.

Major Findings: (1) In order to analyze human cancers for virus genetic information, a variety of human cancer RNAs have been annealed with the DNA product of FeSV, FeLV (grown in human cells), MPMV (Mason Pfizer Monkey Virus grown in human or monkey cells) and MSV. The resulting hybrids were analyzed by hydroxylapatite chromatography and Cs_2SO_4 density gradient centrifugation. Carcinomas of the ovary contained RNA which hybridized with the MSV DNA product. Certain other human cancers contained RNA that hybridized with MPMV DNA and also with FeLV DNA (but not with FeSV or MSV DNA). These data may mean that sequences common to FeLV (grown in human rhabdomyosarcoma cells) and MPMV (grown in human or monkey cells) are present in human cancers.

(2) The 70S RNA genome of the murine sarcoma virus (MSV) and the avian myeloblastosis virus (AMV) contains adenylic-rich sequences (91% adenylic acid) of estimated molecular weight 0,000 to 60,000 (100 to 200 nucleotides) covalently linked to large viral RNA subunits. (A similar finding for Rous sarcoma virus and Rauscher mouse leukemia virus was reported by Lai and Duesberg).

(3) Inhibitors of polymerase molecules which may be used to control the expression of cancer-specific genetic information have been investigated. Of 37 rifamycin SV derivatives with 3-amine substituents, 29 were good inhibitors of the RNA→DNA polymerase activity of RNA tumor viruses, but they also inhibited host cell DNA polymerases. The most potent inhibitors, 3-piperidine derivatives with cyclohexyl or cyclohexylalkyl substituents show an activity of 4 to 5-fold greater than the analogous compounds with benzyl or phenyl substituents. A correlation was found between that ability of 3-cyclic amine rifamycin SV derivatives to inhibit DNA polymerase of RNA tumor viruses and of mammalian cells and to inhibit cell transformation. Of 182 rifamycin SV derivatives with 3-iminomethyl, 3-formyl hydrazone, 3-formyl oxime, 4-desoxy 3, 4-substitutions and 4-desoxy 4-substitutions and rifamycin B derivatives with 4-substitutions, 17 were moderately inhibitory at 20 µg/ml and 10 were strongly inhibitory derivatives. Several derivatives were effective at levels that may be useful for

clinical studies.

(4) At least two size classes of single-stranded RNA molecules sedimenting at 35S and 20S have been isolated from MSV transformed virus-producing mouse and rat cells. These viral RNA species are present both in free and membrane-bound polyribosome fractions of transformed cells. The cryptic state of MSV transformed non virus-producing hamster cells (HT-1) is characterized by two defects: a level of RNA only 2-5% of that present in virus-producing cells and a specific deficiency in 20S RNA. (5) Cells transformed by and continuously producing both sarcoma and leukemia viruses, but not those producing only leukemia viruses, are agglutinated by concanavalin A. It thus appears that the surface changes associated with agglutination are not necessarily associated with the malignant or transformed state of the cell, but may be a function of the expression of certain viral genes associated with sarcoma but not leukemia viruses. (6) Virus-specific RNA transcripts in cells transformed by adenovirus 2 and 7 contain cellular RNA sequences derived from highly reiterated cellular DNA sequences.

Significance to Biomedical Research and the Program of the Institute: Of the five areas listed below, under Proposed Course, the first is important in establishing the relationship of RNA tumor viruses to human cancer; the second is important because of the possible clinical use of these drugs to control the expression of cancer-specific information; the third and fourth are important since they may elucidate the transcription and translation of viral oncogenic information; and the fifth is important to understanding the regulation of macromolecular synthesis in normal and transformed mammalian cells.

Proposed Course: (1) A major effort is well under way on the molecular hybridization of the RNA from a large number of human cancers of defined organ types with DNA products of a variety of RNA tumor viruses, especially those of human and primate origin. (2) The search for and analysis of anti-polymerase drugs will be continued. (3) Analyses of the sequences of virus-specific RNA in non virus-producing transformed cells are continuing. (4) Attempts to isolate the cell-transforming proteins from transformed cells are planned; if demonstrated, their properties and functions will be studied. (5) Basic studies on the mechanism of growth control in normal virus-infected and transformed cells will be performed.

Date Contract Initiated: March 20, 1967

Current Contract Level: $1,200,000

SALK INSTITUTE (PH43-NIH-NCI-E-67-1147)

Title: Temperature-Sensitive Mutants of Polyoma Virus

Contractor's Project Director: Dr. Walter Eckhart

Project Officer (NCI): Dr. Stuart Aaronson

Objectives: To conduct studies on temperature-sensitive mutants of polyoma virus that are defective in functions required for cell transformation.

Major Findings: Temperature-sensitive mutants of polyoma virus have been isolated and characterized in order to identify the viral functions required for cell transformation. The mutants isolated so far can be placed into four or five functional groups, two of which are involved in cell transformation.

The ts3 gene product is required for induction of cellular DNA synthesis, viral DNA synthesis, and for cell surface alteration monitored by agglutination by wheat germ agglutinin or Concanavalin A. It is required for the maintenance of surface properties of transformed cells, and for loss of contact inhibition of DNA synthesis. The ts3 mutant does not complement other ts mutants. This could be explained if ts3 were defective in some aspect of uncoating or early gene expression at high temperature, or if ts3 were defective in a cis-active viral gene function. Temperature shift experiments in the presence and absence of cyclohexamide indicate that the ts3 function is expressed quite early after infection, and once expressed, is not required for the continuation of viral DNA synthesis. The mutant is not blocked; however, at some very early stage of adsorption or penetration, a conclusion that is supported by the observation that ts3 "eclipses" to the same extent, and with the same kinetics, as wild type polyoma during infection at permissive or nonpermissive temperature. It appears that the ts3 gene product is a regulatory protein (which may be part of the virion) whose function is required for the expression of several virus mediated functions during lytic infection, and in the transformed cells.

The gene product of the ts-a mutant is required for viral DNA synthesis and for stable transformation, but not for induction of cellular DNA synthesis or for abortive transformation.

The NG-18 mutant is a host range mutant that is unable to transform hamster or rat cells. NG-18 will genetically complement the "late" mutants that make viral DNA, but do not make infectious virus, at high temperature, and also

mutants in the ts-a group that are defective in transformation at high temperature.

Significance to Biomedical Research and the Program of the Institute: The development of systems of temperature-sensitive mutants of oncogenic viruses represents the application to cancer research of a powerful tool of modern biology. Using the temperature-sensitive mutants, it should be possible to define which viral genes are responsible for the establishment and maintenance of transformation.

Proposed Course: Studies of temperature-sensitive mutants of polyoma virus will continue, as will studies of viral DNA synthesis in isolated cell nuclei, trying especially to promote more efficient initiation of DNA synthesis in the system. The process of infection by viral DNA and DNA-protein complexes will be studied.

Date Contract Initiated: June 5, 1967

Current Contract Level: $120,000

SOUTHERN CALIFORNIA SCHOOL OF MEDICINE, UNIVERSITY OF and CHILDREN'S HOSPITAL OF LOS ANGELES (PH43-NIH-NCI-E-68-1030)

Title: A Comprehensive Field and Laboratory Research Program on the Etiology and Epidemiology of Human Cancer

Contractor's Project Directors: Dr. Murray B. Gardner (USC)
Dr. Robert M. McAllister
(Children's Hospital)

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To mount a multifaceted, highly interrelated program designed to determine the roles of viruses, physical and chemical carcinogens, and other factors in the etiology of human and animal cancer in a natural urban ecology. These studies are carried out at USC School of Medicine and at Children's Hospital of Los Angeles.

Viral studies: Human, pet, and feral animal cancer and fetal materials are under intensive study for RNA tumor virus expression, utilizing all the modern in vitro, as well as in vivo, test systems. Extensive field studies and procurement efforts provide large numbers of tissues derived from cancer patients, genetically defective individuals, and spontaneous and therapeutic abortions. These materials are utilized for in vitro and in vivo biological studies and are subjected to serological, immunological, biochemical, and electron micrographic analyses designed to detect, isolate,

and characterize the RNA viruses and virus-specific antigens associated with naturally occurring animal and human neoplasms.

Epidemiological studies: This program is providing, through hospital record surveys and community questionnaire surveys, up-to-date information of the natural occurrence of human cancer, as it may be influenced by genetic, viral, environmental, or other factors, including exposure to variable smog components in differing ecological areas of Los Angeles County, industrial and household carcinogens, and pets with and without cancer. Other factors such as occupation, aging, genetic defects, smoking, hormone therapy, and immunosuppressants are being studied using classical epidemiological methods combined with virological and serological surveillance.

Environmental studies: This program is concerned with monitoring focal environmental areas for levels of carcinogens and other air pollutants. Materials collected are characterized and supplied to laboratories at USC, as well as to NCI and other SVCP contract programs; e.g., Contract No. NIH-NCI-E-70-2068, for studies to determine the carcinogenic activities of such pollutants in tissue culture and in animals.

Co-carcinogenesis studies: This area is concerned with testing the carcinogenic effects of selected chemical and environmental carcinogens in various inbred and feral strains of mice, hamsters, and cats in vivo, and in selected RNA-virus-infected mammalian tissue culture systems.

Major Findings: A candidate human Type C virus, RD 114, was rescued by Dr. Robert McAllister's group at the Children's Hospital of Los Angeles, working in collaboration with Dr. Murray Gardner (USC) and Dr. Padman Sarma (NCI). Rescue was effected by transplantation of human rhabdomyosarcoma cells (RD cells) into a fetal kitten. The RD 114 virus isolated was, at first, assumed to be a feline leukemia virus, but was found not to contain the FeLV gs-1 or envelope antigens. Further tests in a number of collaborating laboratories have confirmed the RD 114 to be a Type C RNA virus possessing the mammalian group specific antigen (gs-3), but a unique gs-1 antigen.

RNA extracted from RD 114 consisted of 65-70S and 4S components and had reverse transcriptase activity which utilized endogenous 70S RNA or the synthetic polymer poly rA.dT as template.

Preliminary DNA-RNA hybridization experiments in collaboration with Dr. Marcel Baluda at UCLA suggest that the nucleotide

sequence of the 70S RNA of the RD 114 virus is different from the nucleotide sequences of the RNA from two strains of FeLV.

Characterization of the RD 114 virus is continuing in a number of laboratories, including the Viral Carcinogenesis Branch (NCI), Drs. Freeman and Rhim at Microbiolgical Associates (NIH-NCI-E-70-2068), Dr. Bishop at University of California (NIH-NCI-E-71-2147), Dr. Green at St. Louis University (PH43-NIH-NCI-E-67-692), Drs. Gilden, Oroszlan, and Hatanaka at Flow Laboratories (NIH-NCI-E-71-2097), Dr. Rabin at Bionetics (NIH-NCI-E-71-2025), and on site Drs. McAllister, Nicolson, Gardner, Roy-Burman, Rongey, and Rasheed.

RD cells were productively infected with GA-FeLV and in contrast to RD 114 virus, the virus released (RD-FeLV) did have FeLV specific envelope and gs-1 antigens.

RD cells productively infected with KiMSV contained murine virus gs-1 antigen (but not RD 114 gs-1 antigen) and the progeny virus, although not causing focus formation, has a tropism for human and rat cells but not mouse cells.

Biochemical studies of KiMSV and RD-FeLV were continued; in particular, characteristics of their reverse transcriptase reactions.

Transformation and productive infection of a human osteosarcoma cell line with GA-FSV was achieved.

DNA isolated from human adenovirus type 1 was found to be infective for human cells.

A highly efficient method for induction of latent Type C RNA viruses was discovered using BrdU. This method was applied to several human tumor cell lines or primary cultures from human malignancies. Under conditions which regularly led to detection of Type C RNA viruses in rodent or avian culture, no viruses were detected in human cells.

Host dependent antigenic modification of KiMSV was studied on the virus propagated in mouse and rat cells. Type specific antigenicity and in vitro host range (but not the group specific antigenicity) were found to be reversibly host dependent.

Nonproductive lines and monocellular clones transformed by KiMSV were derived from normal rat kidney (NRK) line. They were characterized for virus production by focus assay, COMUL test, electron microscopy, and H^3-uridine incorporation and found to be free of Type C viruses.

High molecular weight (70S) RNA isolated form KiMSV induced transformation in NRK cells. Transformed cells did not release detectable virus but did contain rescuable viral genome.

An inhibitor, very likely an antibody, directed against the DNA polymerase of the mammalian Type C RNA tumor viruses, was detected in the sera of cats with spontaneous tumors or FSV induced sarcomas.

Twenty-three nontransforming, mostly N-tropic, Type C isolates were recovered in vitro from wild mouse embryo cells, breast tissue, and 3-MC-induced sarcomas. Eighteen of these isolates were obtained from mice of one trapping area in which a very high prevalence (80-90%) of Type C virus activity was detectable. This colony is interesting and may reflect a peculiar segregation of genes of a very atypical nature since all of a dozen other normal wild mice populations were negative for this virus.

Two sarcoma viruses have been isolated in vitro, one of which has so far produced sarcomas in vivo in wild mice. Both sarcoma isolates were found to grow better at 32° (close to normal mouse body temperature), than 37°, a finding which may have potential importance in other tissue culture systems as well. Most virus-negative wild mouse cell cultures were resistant to infection with exogenous Type C virus of wild mouse origin.

Type B virus particles have been found by EM in over 50% of the breast biopsies from pregnant mice trapped throughout Los Angeles County; thus, wild mice provide a natural source of the difficult to obtain mammary tumor virus particles.

Of the dozen separate wild mouse colonies under study, one revealed a high level of infection with polyoma. In view of the cancer-inducing properties of polyoma in experimental systems, this colony is of particular interest to determine what effect, if any, polyoma has on cancer incidence, age at onset, effect of carcinogens, etc., in its natural host.

An excellent correlation between the detection of FeLV gs-1 antigen (using guinea pig sera prepared against this purified antigen by Dr. R. Gilden of Flow Laboratories, Contract No. NIH-NCI-E-71-2097) and Type C particles by EM has been obtained in cats with spontaneous lymphoma, unexplained severe anemia, and in normal cats. Cats with unexplained severe anemia had as much, or even more (80-90%), Type C virus expression than cats with spontaneous lymphoma. Virus particles and gs-1 antigen were detected in about 10% of normal cats. Cultures obtained from tissues of 7 of 26

different fetal cats contained demonstrable Type C virus activity in early subpassage.

Eighty-three hospitals, representing more than 70% of the 30,000 eligible acute hospital beds in Los Angeles County, are participating in the Cancer Surveillance Program. Approximately 10,000 current cancer cases have been collected, abstracted, recorded on computer tape, and are being analyzed. Field studies of several types of cancers occurring in populations at Leisure World and several other seroepidemiological studies are under way. Epidemiologic studies are in progress on (a) the possible role of the putative human Type B particle in the pathogenesis of human breast cancer; (b) Hodgkin's disease and EBV; (c) young genital tract cancer, particularly in relation to maternal stilbesterol; and (d) human lymphocyte antigen phenotypes in relation to human cancer.

Samples of air pollutants have been collected. Crude extracts and some subfractions have been provided to Drs. Freeman and Rhim at Microbiological Associates (Contract No. NIH-NCI-E-70-2068), Rasheed at USC (Contract No. PH43-NIH-NCI-E-68-1030), and Weiss at USC (Contract No. NIH-NCI-E-72-2032). Most of the fractions studied in vitro have been found to have some transforming activity. Samples of these extracts are available to other interested investigators.

Significance to Biomedical Research and the Program of the Institute: This program searches for causes of human, pet, and other animal cancers in a natural ecology, utilizing (1) experimental animal systems; (2) basic viral and chemical carcinogenesis studies; and (3) epidemiological profiles of cancer incidence in Los Angeles Area humans and animals in relation to exposure, and to environmental carcinogens.

In addition, the program continues as a prime resource for supplying human and animal materials to the Viral Oncology in-house and SVCP contract programs, particularly to Microbiological Associates (NIH-NCI-E-70-2068), Flow Labs., Inc. (NIH-NCI-E-71-2097), St. Louis University (PH43-NIH-NCI-E-67-692), University of California (Naval Biomedical Research Laboratories [PH43-NIH-NCI-E-63-13]), and the California State Department of Public Health (PH43-NIH-NCI-E-68-997).

Proposed Course: Continuation of (1) studies with the candidate human Type C virus, RD 114, (2) studies of mouse and cat Type C viruses, (3) co-carcinogenesis studies, (4) epidemiological studies of factors influencing cancer incidence, (5) characterization of air pollutants. (6) Procurement, growth, and distribution of human and animal materials.

Date Contract Initiated: June 26, 1968

Current Contract Level: $2,499,040

SOUTHERN CALIFORNIA SCHOOL OF MEDICINE, UNIVERSITY OF (NIH-NCI-E-72-2032)

Title: Conditional Lethal Mutants of RNA Tumor Viruses

Contractor's Project Director: Dr. Peter K. Vogt

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: (1) Conditional lethal mutants of avian sarcoma viruses will be isolated. (2) All mutants will be tested for leakiness and genetic stability. (3) The reproductive and cell transforming abilities of the mutants will be delineated under permissive and nonpermissive conditions. (4) The location of the conditionally lethal step in the infectious cycle will be determined. (5) The epidemic of chicken tumors occurring in Southern California in the Autumn of 1971 will be studied.

Major Findings: The contractor has been collaborating with Dr. Gardner's group (Contract No. PH43-NIH-NCI-E-68-1030) in studies of a major epidemic of tumors which occurred in chicken populations of Southern California during the Fall of 1971. The epidemic appeared to coincide with the delivery of the first batches of chickens which had received the new Marek's disease vaccine. In such chicken populations condemnations due to Marek's disease leukosis had declined; however, at the same time a rise in the incidence of solid tumors occurred. These tumors included sarcomas, Wilms tumors, and hemangiomas.

Fresh chicken tumor specimens were obtained and put in culture with normal chicken fibroblasts serving as feeder layers. In about half of the tumor cultures a cytopathic rounding of the feeder cells occurred and eventually spread through the entire culture causing cell death. The effect could be transferred to normal chicken and quail fibroblasts with cell-free supernatants of the infected cultures. However, freezing and thawing of these supernatants led to almost complete loss of activity. Low multiplicities of infection resulted in the formation of foci consisting of rounded cells not unlike those transformed by Rous sarcoma virus. However, these cells failed to pile up and lacked the capacity for prolonged growth. Infected cultures were studied by electron

microscopy in Dr. Gardner's group. Both herpesvirus and Type C particles were seen. Complement fixation tests of the tumors (carried out in Dr. R. Huebner's laboratory) indicated that the group specific antigen of avian RNA tumor viruses is present in the tumor tissue in greater concentration than in normal tissues.

The data available so far are compatible with the suggestion that the cytopathic effect seen in culture is caused by a herpesvirus. Whether this virus is also responsible for the tumor formation in vivo is not known at the moment. The spectrum of tumors seen in the chicken population (hemangiomas, sarcomas, and Wilms tumors) is more indicative of an avian RNA tumor virus than of Marek's disease herpesvirus. Experiments are in progress to inactivate the herpesvirus component in tissue culture samples selectively and to identify the surviving Type C particles. Infected cultures have also been frozen and are available to qualified investigators.

Significance to Biomedical Research and the Program of the Institute: RNA tumor viruses are oncogenic under natural conditions and probably occur in all vertebrates including man. It is therefore important to understand their mode of replication and the mechanism of their oncogenic action. A difficulty has been in differentiating between cellular and viral macromolecular synthesis. Conditional lethal mutants could alleviate this situation because accumulation of viral precursors will occur under nonpermissive conditions. The levels of viral material reached in the cell may thus become detectable with currently available biochemical techniques. Conditional lethal mutants will also be useful in delineating the sequence of viral functions during infection and in pointing out patterns of interdependence in such functions.

Even more important than the contribution temperature-sensitive mutants may make to our understanding of virus replication is their potential value for the analysis of neoplastic transformation. Inability of temperature-sensitive mutants to transform under nonpermissive conditions would strongly implicate the viral genome in the process of transformation. Transformation negative mutants which can transform at 35° but are unable to perpetuate transformation at 41° will be significant in the identification of specific viral products and functions which are needed for the maintenance of the neoplastic state. Preliminary observations with avian sarcoma viruses indicate that such types of mutants can be obtained.

Proposed Course: Since the work being currently done on the epidemic of chicken tumors is an interim project, it is

planned to conclude this work by characterizing the RNA tumor virus components obtained from the tumors; work will continue further with these agents only if they are able to produce transformation in vitro and can be used directly in mutant work.

It is anticipated that very shortly the equipment needed for the isolation and characterization of new temperature-sensitive mutants will be delivered, and the work on temperature-sensitive mutants will begin.

Most work will be carried out with avian sarcoma virus B77. Chick embryo fibroblast cultures infected with a high multiplicity of virus become completely transformed within a few days and actively produce virus. Such cells will be exposed to 5-azacytidine (1-25 µg per ml) which has been shown to cause mutations in replicating RNA viruses. Virus stocks grown in the presence of the mutagen for 24 hours will be harvested and studied for the presence of conditional lethal mutants.

As in previous work, incubation at 41°C will be chosen as nonpermissive condition, and 35°C will constitute the permissive environment. All temperature-sensitive mutants will be classified according to the viral product or function which is missing at the nonpermissive temperature. Products and functions which will be tested in this initial classification are (1) neoplastic transformation of the infected cell, (2) production of infectious or noninfectious viral particles, (3) synthesis of type-specific antigen, (4) synthesis of group-specific antigen.

The approximate location of the temperature-sensitive step in each conditional lethal mutant will be determined by shifting the temperature of incubation at various times after infection from 35-41° or vice versa. It will be possible to divide the mutants in two categories, one having an early temperature-sensitive step and the other showing late temperature sensitivity.

Date Contract Initiated: October 15, 1971

Current Contract Level: $256,000

STANFORD UNIVERSITY (NIH-NCI-E-69-2053)

Title: (A) Procurement, Processing, Storage, Distribution and Study of Human Tumor cell Cultures; and (B) Operation of a Central Mycoplasma Diagnostic Laboratory

Contractor's Project Director: Dr. Leonard Hayflick

Project Officer (NCI): Dr. Stuart Aaronson

Objectives: Part A is for the procurement, processing, and distribution to SVCP contractors of viable, characterized human tumor cell cultures obtained from biopsy or surgical removal at hospitals in the San Francisco Bay Area. (Serum samples are collected simultaneously.) In addition research studies are directed toward the detection of a viral genome in these cells. Part B serves as a central diagnostic facility for the detection and identification of mycoplasma contamination in virus preparations, sera, cell cultures, and clinical materials submitted by other SVCP contractors. Upon request, identification of isolates is made as to species; and mycoplasma antigens are distributed to those investigators requiring these materials.

Major Findings: (1) Over 68 human tumors were cultivated during the year. All viable cultures have been photographed, and from 3 to 11 ampules of tumors have been stored in liquid nitrogen. (2) All tumor culture data, patient history, and cell storage information is now stored in a computer. (3) Thirteen human tumor cell populations have been examined by electron microscopy for Type C particles with negative results. (4) With cytochalasin B, enucleation of the entire culture of human normal (WI-38) and cancer cells has been achieved, thus paving the way for hybridization and genome rescue experiments. (5) The use of antilymphocyte-serum (ALS) treated mice in assessing the malignancy of human tumor cells is continuing. ALS has now been prepared against the C57/L strain of mouse (one of the most "switched-off" strains in terms of Type C particles), in which future studies will be done. (6) Treatment of WI-38, human embryo cells, and human amnion with benzo-(a)-pyrene or methyl-cholanthrene or FeSV does not result in a malignant transformation. (7) During 1971, 3,156 samples were received from SVCP laboratories and were tested for mycoplasma contamination; 358 were positive. (This represents the largest number of mycoplasma samples received in any year since the inception of this contract 7 years ago.) (8) Studies continue on the growth characteristics of mycoplasmas in an effort to understand the interaction of mycoplasmas with cells cultured in vitro and the effects of new mycoplasma inhibitors with the goal of developing a control for mycoplasma contamination.

Significance to Biomedical Research and the Program of the Institute: The mycoplasma diagnostic facility is a testing and monitoring service available to all SVCP contractors and Viral Oncology intramural staff. Many of the most important viral specimens, cell cultures, sera, etc., used in the Viral Oncology Program are sent to this facility for mycoplasma testing. In addition the contractor is growing

human tumor cells in vitro as a resource for other SVCP contractors and for the purpose of his own research on transformation of cells by oncogenic viruses. The system of C57/L mice treated with anti-lymphocyte serum will be used to tell whether cells from neoplastic tissues, being grown in vitro, are "tumor cells or fibroblasts."

Proposed Course: (1) Continuation of collection, cultivation, characterization, and storage of human tumor cells, as well as mycoplasma testing of samples received from other laboratories. (2) Continuation of studies on: (a) effects of various chemical carcinogens on normal human cells in vitro, alone and in combination with oncornaviruses; (b) discrimination by C57/L mice treated with antilymphocytic sera (ALS) between human normal and cancer cells; (c) cell fusion, hybridization and keterokaryon formation in vitro using cytochalasin B and Sendai virus. (d) Detection of gs antigen in human embryonic tissue and tumor cells. (e) Electron microscopy of cultured human tumor cells. (f) Attempts to transform a variety of normal human cells with RNA tumor viruses.

Date Contract Initiated: June 19, 1969

Current Contract Level: $178,447

WASHINGTON, UNIVERSITY OF (NIH-NCI-E-71-2171)

Title: Studies on Tumor-specific Transplantation Antigens

Contractor's Project Directors: Dr. Karl Erik Hellström
Dr. Ingegerd Hellström

Project Officer (NCI): Dr. Charles W. Boone

Objectives: To detect and characterize tumor-specific antigens, plus serum-mediated and cell-mediated immune responses to these tumor-specific antigens, in human tumors.

Major Findings: (1) Human neoplasms derived from the same tissue had been previously shown, by the contractor, to have common tissue type specific tumor associated antigens since lymphocytes from a given cancer patient will inhibit his own tumor cells in tissue culture and also tumor cells of other patients of the same histologic type, but not of different histologic types. Evidence was now found for the existence of analogous antigens common to both rat bladder papillomas and carcinomas, and for such antigens common to mouse bladder carcinomas. Murine bladder tumors may thus be suitable for an investigation of immune reactions against tumor associated antigens of the tissue specific type. Rats

immunized with syngeneic urinary bladder papillomas, then challenged by insertion of a methylcholanthrene pellet into the bladder, were found to develop fewer primary bladder tumors than rats immunized with normal bladder tissue.

(2) The contractor had previously shown that human tumors of the same histologic type have common blocking antibodies since the serum from a given patient will block the tumor-destructive effect of his own lymphocytes for his own tumor and also for tumors of the same histologic type from other patients. It was now shown that in addition to sera a similar blocking activity can be eluted at pH 3.1 from human tumor tissues, obtained at surgery (seminomas and osteogenic sarcomas), and from tumor cells growing in ascites and pleural effusions (carcinomas of endometrium, breast, and ovary). A high (MW above 100,000) and a low (MW below 100,000) molecular weight fraction can be separated from the eluates by ultrafiltration. Neither of these fractions could block lymphocyte mediated cytotoxicity in vitro when tested by itself, while a 1:1 mixture of them could. Blocking was also obtained when the tumor cells were first exposed to the high and then to the low molecular weight fractions, but not when the sequence was reversed. The observations obtained are analogous to previous findings in animal tumor systems and provide evidence that tumors growing in human patients are coated by "blocking factors."

(3) Blocking material, in the form of sera of rats carrying progressively growing polyoma tumors and low pH eluates of polyoma tumor tissue inoculated into polyoma isografted rats, cause an enhanced tumor growth.

(4) Sera from all of seven tumor patients tested (one with melanoma, one with colonic carcinoma and five with breast carcinoma), who had become clinically tumor-free, could "unblock" the blocking effect of sera from patients bearing tumors of the respective types, thus making it possible for immune lymphocytes to kill cultivated neoplastic cells in the combined presence of the "unblocking" and blocking sera.

(5) "Unblocking" antibodies can be produced by immunization of BCG primed rats or rabbits with tumor cells. These antibodies can counteract the effect of specific blocking sera and tumor eluate in vitro. When inoculated into rats which had previously received a polyoma tumor isograft, unblocking serum was found to inhibit the appearance of (or counteract) the blocking activity of serum in four of five rats. Tumors in these four rats grew progressively for 10-12 days and then completely regressed.

Significance to Biomedical Research and the Program of the Institute: This important and productive study of human and animal tumor antigens, as well as cell-mediated and humoral immune responses to the antigens, has already given, and promises to continue to give, insights to the understanding of the body's immune response to tumors, and ultimately may lead to immunotherapy of established tumors in man.

Proposed Course: Continuation of screening of human and animal tumors for tumor-specific transplantation antigens. Continuation of in vitro studies of cell-mediated and humoral immune responses against TSTA, including "blocking" and "unblocking" factors, and attempts at immunotherapy of established tumors.

Date Contract Initiated: April 14, 1969

Current Contract Level: $90,000

WEIZMANN INSTITUTE OF SCIENCE (NIH-NCI-E-69-2014)

Title: Study of Virus-induced Tumor-specific Transplantation Antigens

Contractor's Project Director: Dr. Leo Sachs

Project Officer (NCI): Dr. Charles W. Boone

Objectives: To investigate the differences between the structure of the surface of normal cells and of cells transformed by viral and non-viral carcinogens by studying the differential binding of plant lectins.

Major Findings: Concanavalin A (Con. A) agglutinates transformed cells, but it only agglutinates normal cells after they have been treated with tyrpsin. Only about 15% of the cell surface is occupied by Con. A molecules at saturation.

Transformed cells were agglutinated by Con. A at 24°C but not at 4°C. Agglutination of transformed cells by wheatgerm agglutinin, which binds to N-acetyl-D-glucosamine-like sites, and by soybean agglutinin, which binds to N-acetyl-D-galacyosamine-like sites, was not temperature-sensitive.

Changes in the structure of the cell surface were found in the ascites form of a Moloney virus-induced lymphoma, and in cells transformed by polyoma virus, SV40, Rous sarcoma virus, and the chemical carcinogen, dimethylnitrosamine.

Cells of a Moloney virus-induced lymphoma (YAC) were agglutinated by Con. A, wheatgerm and soybean agglutinins. However, only Con. A showed a marked toxic effect on YAC cells in vitro and in vivo. In vitro incubation with 125 µg Con. A for 24 hours lysed 95% of the cells. Intraperitoneal injection of 1 mg Con. A at 1 hour, 2 days, and 5 days after intraperitoneal inoculation of 10^2 YAC cells into adult mice resulted in an inhibition of tumor formation in 70%, 50%, and 20% of the animals, respectively.

Amino acid and carbohydrate transport in normal and malignant transformed hamster cells was studied after equal binding Con. A to the cell surface. The transport of a number of amino acids was inhibited after Con. A binding in the transformed cells but not in the normal cells (e.g., L-leucine, L-arginine, L-glutamic acid, L-glutamine, cycloleucine, and α-aminoisobutyric acid). Transport of D-glucose and D-galactose was more inhibited by Con. A in transformed than in normal cells. There was no effect on the transport of L-fucose or 3-0-methyl-D-glucose in either normal or transformed cells.

Significance to Biomedical Research and the Program of the Institute: Compounds that interact differentially with the surface of normal and tumor cells are of value in elucidating the chemical nature of the differences in the surface that are associated with cell malignancy. They are also of potential value for tumor chemotherapy.

Proposed Course: The potential therapeutic value of Con. A will be further explored in three main directions: (1) Chemical modifications of Con. A to attempt to improve the degree of previously shown differential toxic effect on normal and tumor cells. (2) Studies of what enables Con. A to produce cell toxicity (how it differs from other lectins which bind but do not kill). (3) Further studies of the ability of Con. A to induce the activation of lymphocytes.

Date Contract Initiated: April 22, 1969

Current Contract Level: $51,600

WISTAR INSTITUTE OF ANATOMY AND BIOLOGY (NIH-NCI-E-71-2092)

Title: Extraction and Characterization of Virus-induced Transplantation Antigen and Rescue of Virus from Sarcomas and Leukemias

Contractor's Project Director: Dr. Anthony J. Girardi

Project Officer (NCI): Dr. Charles W. Boone

Objectives: To extract and characterize tumor-specific transplantation antigens induced by selected DNA and RNA tumor viruses.

Major Findings: (1) In cooperation with Dr. Berge Hampar (NCI), the peroxidase staining technique for localization of murine gs antigen is being evaluated. The technique is both more sensitive and more specific than the fluorescent antibody technique. (2) Examination of early fetal hamster tissue for antigens shared with SV40-induced tumor cells has confirmed Coggin's findings that such a common antigen(s) exists, but does not support the concept that it is the important transplantation type antigen since it protects only male animals. His findings have been extended to show the embryos of primaparous females were effective immunogens against SV40 tumorigenesis while those from multiparous females were not. (3) Rescue attempts have been initiated employing human sarcomas and leukemias in a variety of combinations designed to activate latent viral genomes. These include co-cultivation and fusion with either BPL inactivated Sendai virus or lysolecithin.

Significance to Biomedical Research and the Program of the Institute: The treatment of cancer by immunologic methods has been an attractive hypothesis for decades, but it is only recently that new and fundamental discoveries in immunobiology have made cancer immunotherapy a real possibility. Many tumors possess individually distinct transplantation antigens against which the host mounts an immune response. The transplantation antigens of most virus-caused tumors in different animal species are the same for a given virus. The work being conducted by the contractor is part of a larger effort of the SVCP to isolate and test virus-induced tumor-specific transplantation antigens in animal model systems.

Proposed Course: (1) Improve use of the peroxidase reaction technique as a routine assay method for gs antigens and for intracellular localization of reverse transcriptase. (2) Study of the relationship of fetal antigen to transplantation antigen. (3) Attempt to rescue human oncogenic viruses using co-cultivation, fusion, and chemical activation with BrdU and IdU. (4) Continuation of attempts to prevent spontaneous tumors of mice by immunological techniques using direct immunization with vaccines, non-specific stimulation, and abrogation of tolerance to isogeneic tissues. (5) Continuation of attempts to immunize against neoplasia induced by known laboratory strains of oncorna viruses. (6) Continuation of attempts to purify transplantation antigen extracted by

high molarity salt solutions or neuraminidase treatment, and characterization of the isolated component(s).

Date Contract Initiated: February 1, 1971

Current Contract Level: $115,400

THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY
NIH-NCI-E-69-2007)

Title: Isolation and Purification of Transplantation Antigens

Contractor's Project Director: Dr. Donald F. H. Wallach

Project Officer (NCI): Dr. Charles W. Boone

Objectives: To investigate the biochemical and antigenic changes which occur at the cell surface during and after transformation by oncogenic viruses.

Major Findings: This program continued to search for new proteins in monkey kidney cells during early stages of permissive, lytic infection by the oncogenic DNA virus SV40. It relied heavily upon various methods of cell fractionation and upon polyacrylamide gel electrophoresis for visualizing the protein components of isolated cell fractions. Repeated experiments produced consistent protein patterns for uninfected cell fractions. The latest and best-controlled experiments show no new gel bands visible in fractions of infected cells if gels are stained in the Coomasie blue or Stains-All dyes. However, if the cells are labeled with glucosamine-1-^{14}C, microsome and microsome wash fractions of infected cells display at least one confirmed ^{14}C band not found in comparable fractions of control cells. The microsome "washes" were not innocuous rinses, but removed materials whose protein spectrum was different from the soluble fraction and whose ^{14}C specific activity was considerably higher. Inasmuch as by far the highest specific activity of any cellular component was found in the most buoyant microsome fraction, the activity of the material in the washes suggests that it is rich in membrane glycoprotein or in an export material which can be found on cell surface, or both. This "new" glucosamine-labeled component of microsomes from infected cells will be investigated further, using non-SVCP support.

Significance to Biomedical Research and the Program of the Institute: N/A

Proposed Course: This contract was terminated in September, 1971.

Date Contract Initiated: March 12, 1969

Current Contract Level: None

SALK INSTITUTE (NIH-NCI-E-72-3207)

Title: Growth Regulation of Normal and Transformed Cells and Immunological Approaches to Tumor Rejection and Prevention

Contractor's Project Director: Dr. Edwin S. Lennox

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: (1) Study how the surface of tumor cells differs from that of normal cells. (2) Attempt immunological prevention and rejection of tumors. (3) Study growth regulation of normal and cancer cells, as directed by serum factors.

Major Findings: This is a new contract and major findings have not yet been reported.

Significance to Biomedical Research and the Program of the Institute: The proposed contract focuses on the antigenic properties of cancer cells which are undoubtedly involved in the phenomena of unrestrained growth and metastasis of tumors. Projects A, C, and D work respectively towards an ultimate goal of enhanced immune rejection of tumors, regulation of tumor growth, and a chemical anti-tumor vaccine, any of which would be a major step forward in the control of human cancer.

Proposed Course: Project A. (Drs. Dulbecco & Lennox) Tumor cell lines with altered antigenic characteristics will be derived to test their ability to induce immune rejection of the parent tumor.

Project B. (Dr. Nicolson) The amount and distribution of cell surface antigens in normal and tumor cells will be studied using radioimmune assay, cytotoxic methods and especially EM visualization using ferritin-conjugated antibodies and plant lectins.

Project C. (Dr. Holley) The difference in the regulation of growth of normal and malignant cells by serum factors will be studied.

Project D. (Dr. Shier) Synthetic antigens will be prepared with chemical structure similar to that of the receptors on tumor cells responsible for agglutination with various plant lectins, and these antigens will be tested as immunogens to elicit anti-tumor cellular responses.

Date Contract Initiated: March 6, 1972

Current Contract Level: $398,640

ETIOLOGY AREA

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TABLE OF RATIOS OF CONTRACT TO
INTRAMURAL EFFORT BASED ON
CURRENT LEVEL OF EXPENDITURE
JUNE 30, 1972

	OSDE	DEMOGRAPHY	CARCINOGENESIS	VIRAL ONCOLOGY	TASK FORCES	TOTAL
Total	1,050,000	8,104,000	22,208,000	46,305,000	4,153,000	81,820,000
Contracts	--	5,940,000	19,071,000	40,133,000	3,830,000[1]	68,974,000
Intramural*	1,050,000	2,164,000	3,137,000	6,172,000	323,000[2]	12,846,000
Ratios: Contract to Intramural	--	2.7:1	6.1:1	6.5:1	11.8:1	5.4:1

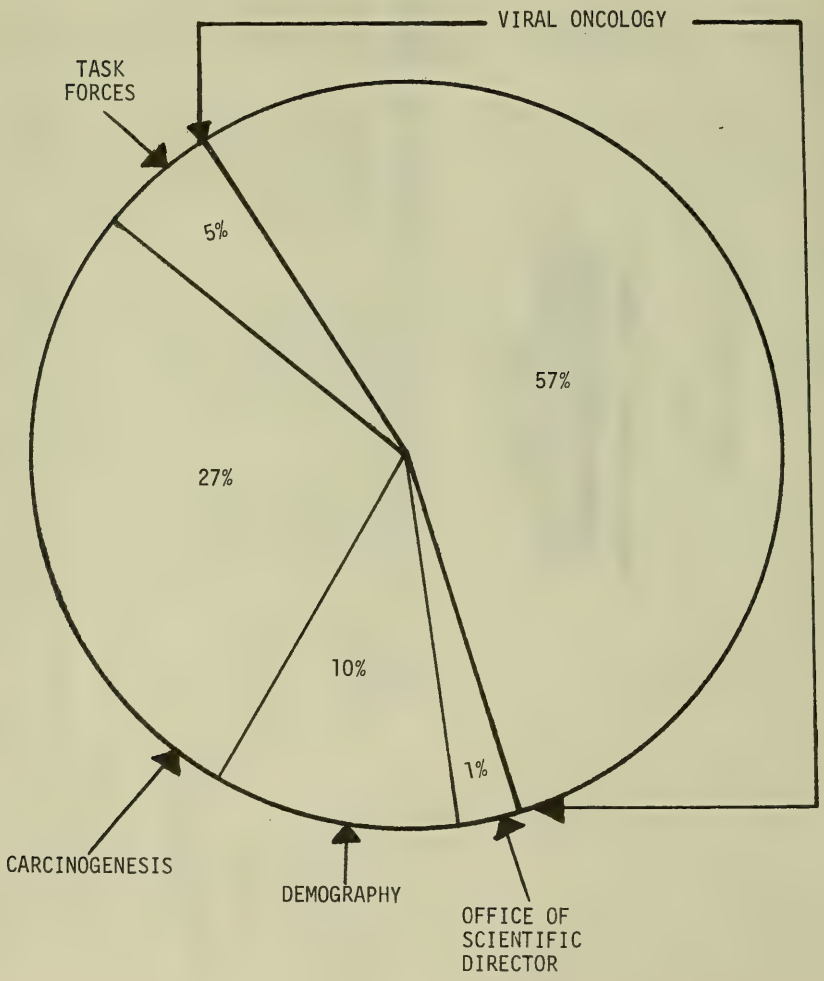
*Includes costs of managing contract activities.

[1]Includes $2,196,000 for Lung Cancer, $400,000 for Large Bowel, $500,000 for Pancreas (Carcinogenesis area); and $734,000 for Breast Cancer (Viral Oncology area).

[2]Direct operations for Lung Cancer.

NATIONAL CANCER INSTITUTE
ETIOLOGY AREA

CURRENT DISTRIBUTION OF FUNDS - FISCAL YEAR 1972



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Bryan, W. Ray	Scientific Coordinator in Viral Oncology
Depue, Robert H., Jr.	Scientist Director
Clausen, Harry J.	Biologist

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Velthuis, Robert, Jr.	Administrative Officer, Viral Oncology
Vennetti, James C.	Administrative Officer, Demography
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Office of Associate Scientific Director for Demography

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Myers, Max H.	Res. Math. Statistician
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Cramer, Daniel W.	Surgeon
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Heise, Herman W.	Statistician (Health)
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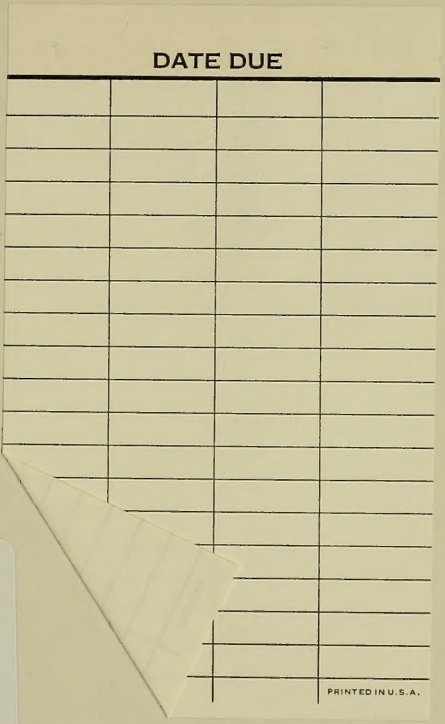

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